

# Organometallic Palladium Reagents for Cysteine Bioconjugation.

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*Supporting Information*

## 1. General Information

### General Reagent Information

*Tris*(2-carboxyethyl)phosphine hydrochloride (TCEP•HCl) was purchased from Hampton Research (Aliso Viejo, CA). 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), D-Biotin, Fmoc-Rink amide linker, Fmoc-L-Gly-OH, Fmoc-L-Leu-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Ala-OH, Fmoc-L-Cys(Trt)-OH, Fmoc-L-Gln(Trt)-OH, Fmoc-L-Asn(Trt)-OH, Fmoc-L-Glu(*O**t*Bu)-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Phe-OH, Fmoc-L-Ser(*t*Bu)-OH, Fmoc-L-Thr(*t*Bu)-OH, Fmoc-L-Tyr(*t*Bu)-OH, and Fmoc-L-His(Trt)-OH were purchased from Chem-Impex International (Wood Dale, IL). Aminomethyl polystyrene resin was prepared according to an in-house protocol.<sup>1</sup> Peptide synthesis-grade *N,N*-dimethylformamide (DMF), dichloromethane (DCM), diethyl ether, HPLC-grade acetonitrile, and guanidine hydrochloride were obtained from VWR International (Philadelphia, PA). Aryl halides and aryl trifluoromethanesulfonates were purchased from Aldrich Chemical Co., Alfa Aesar, or Matrix Scientific and were used without additional purification. All deuterated solvents were purchased from Cambridge Isotopes and used without further purification. All other reagents were purchased from Sigma-Aldrich and used as received.

All reactions with peptides, proteins, and antibodies were set up on the bench top and carried out under ambient conditions. For procedures carried out in the nitrogen-filled glovebox, the dry degassed THF was obtained by passage through activated alumina columns followed by purging with argon. Anhydrous pentane, cyclohexane, and acetonitrile were purchased from Aldrich Chemical Company in Sureseal® bottles and were purged with argon before use.

### **General Analytical Information**

All small-molecule organic and organometallic compounds were characterized by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, and IR spectroscopy, as well as elemental analysis (unless otherwise noted).  $^{19}\text{F}$  NMR spectroscopy was used for organometallic complexes containing a trifluoromethanesulfonate counterion.  $^{31}\text{P}$  NMR spectroscopy was used for characterization of palladium complexes. Copies of the  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ , and  $^{19}\text{F}$  NMR spectra can be found at the end of the Supporting Information. Nuclear Magnetic Resonance spectra were recorded on a Bruker 400 MHz instrument and a Varian 300 MHz instrument. Unless otherwise stated, all  $^1\text{H}$  NMR experiments are reported in  $\delta$  units, parts per million (ppm), and were measured relative to the signals of the residual proton resonances  $\text{CH}_2\text{Cl}_2$  (5.32 ppm) or  $\text{CH}_3\text{CN}$  (1.94 ppm) in the deuterated solvents. All  $^{13}\text{C}$  NMR spectra are measured decoupled from  $^1\text{H}$  nuclei and are reported in  $\delta$  units (ppm) relative to  $\text{CD}_2\text{Cl}_2$  (54.00 ppm) or  $\text{CD}_3\text{CN}$  (118.69 ppm), unless otherwise stated. All  $^{31}\text{P}$  NMR spectra are measured decoupled from  $^1\text{H}$  nuclei and are reported relative to  $\text{H}_3\text{PO}_4$  (0.00 ppm).  $^{19}\text{F}$  NMR spectra are measured decoupled from  $^1\text{H}$  nuclei and are reported in ppm relative to  $\text{CFCl}_3$  (0.00 ppm) or  $\alpha,\alpha,\alpha$ -trifluorotoluene (-63.72 ppm). All FT-IR spectra were recorded on a Thermo Scientific – Nicolet iS5 spectrometer (iD5 ATR – diamond). Elemental analyses were performed by Atlantic Microlabs Inc., Norcross, GA.

### **LC-MS Analysis**

LC-MS chromatograms and associated mass spectra were acquired using Agilent 6520 ESI-Q-TOF mass spectrometer. Solvent compositions used in the majority of experiments are 0.1% TFA in  $\text{H}_2\text{O}$  (solvent A) and 0.1% TFA in acetonitrile (solvent B). The following LC-MS methods were used:

**Method A** LC conditions: Zorbax SB  $\text{C}_3$  column: 2.1 x 150 mm, 5  $\mu\text{m}$ , column temperature: 40  $^\circ\text{C}$ , gradient: 0-3 min 5% B, 3-22 min 5-95% B, 22-24 min 95% B, flow rate: 0.8 mL/min. MS conditions: positive electrospray ionization (ESI) extended dynamic mode in mass range 300 – 3000  $m/z$ , temperature of drying gas = 350  $^\circ\text{C}$ , flow rate of drying gas = 11 L/min, pressure of nebulizer gas = 60 psi, the capillary, fragmentor, and octupole rf voltages were set at 4000, 175, and 750, respectively.

**Method B** LC conditions: Zorbax SB  $\text{C}_3$  column: 2.1 x 150 mm, 5  $\mu\text{m}$ , column temperature: 40  $^\circ\text{C}$ , gradient: 0-2 min 5% B, 2-11 min 5-65% B, 11-12 min 65% B, flow rate: 0.8 mL/min. MS conditions are same as *Method A*.

**Method C** LC conditions: Zorbax SB C<sub>3</sub> column: 2.1 x 150 mm, 5 μm, column temperature: 40 °C, gradient: gradient: 0-2 min 5% B, 2-10 min 5-95% B, 10-11 min 95% B, flow rate: 0.8 mL/min. MS conditions are same as *Method A*.

Data were processed using Agilent MassHunter software package. Deconvoluted masses of proteins were obtained using maximum entropy algorithm.

LC-MS data shown were acquired using Method A, unless otherwise noted; Y-axis in all chromatograms shown in supplementary figures represents total ion current (TIC); mass spectrum insets correspond to the integration of the TIC peak unless otherwise noted.

### **Determination of Reaction Yields**

All reported yields were determined by integrating TIC spectra. First, the peak areas for all relevant peptide-containing species on the chromatogram were integrated using Agilent MassHunter software package. Since no peptide-based side products were generated in the experiments, the yields shown in Table 2 were determined as follows: %yield =  $S_{pr}/S_{total}$  where  $S_{pr}$  is the peak area of the product and  $S_{total}$  is the peak area of combined peptide-containing species (product and starting material). The yield of the stapled peptide **P3-A** was calculated as follows: %yield =  $k \cdot S_{pr}/S_{st}$  where  $S_{pr}$  is the peak area of the reaction product,  $S_{st}$  is the peak area of a known amount of purified product, and  $k$  equals to the ratio of the known amount of standard divided by the initial amount of starting material. For peptide stability experiments the conversion was calculated as following: %remaining peptide =  $S_t/S_0$  where  $S_t$  is the peak area of the corresponding cysteine conjugate at time  $t$ , and  $S_0$  is the peak area of the cysteine conjugate at time 0.

## **2. X-Ray Structure Determination**

Low-temperature diffraction data ( $\varphi$ - and  $w$ -scans) was collected on a Bruker-AXS X8 Kappa Duo diffractometer coupled to a Smart Apex2 CCD detector with Mo  $K\alpha$  radiation ( $\lambda = 0.71073$  Å) from an  $I\mu S$  micro-source for the structure of compound **1A-OTf**. The structure was solved by direct methods using SHELXS<sup>2</sup> and refined against  $F^2$  on all data by full-matrix least squares with SHELXL-97<sup>3</sup> using established refinement techniques.<sup>4</sup> All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included into the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the  $U$  value of the atoms they are linked to (1.5 times for methyl groups). All disordered atoms were refined with the help of similarity restraints on the

1,2- and 1,3-distances and displacement parameters as well as rigid bond restraints for anisotropic displacement parameters unless otherwise noted below.

X-ray quality crystals of **1A-OTf**•CH<sub>3</sub>CN were obtained by vapor diffusion of an Et<sub>2</sub>O/CH<sub>3</sub>CN solution of **1A-OTf** with pentane. Under these conditions, a molecule of CH<sub>3</sub>CN coordinates to palladium in **1A-OTf**, displacing the trifluoromethanesulfonate anion. The resulting **1A-OTf**•CH<sub>3</sub>CN crystallizes in the monoclinic space group *P2<sub>1</sub>* with one molecule in the asymmetric unit.

**Table S1.** Crystal data and structure refinement for **1A-OTf**•CH<sub>3</sub>CN.

Empirical formula	C <sub>40</sub> H <sub>53</sub> F <sub>3</sub> NO <sub>5</sub> PPdS	
Formula weight	854.26	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2 <sub>1</sub>	
Unit cell dimensions	a = 10.5958(3) Å	α = 90°.
	b = 17.8048(6) Å	β = 98.6068(10)°.
	c = 10.9802(4) Å	γ = 90°.
Volume	2048.15(12) Å <sup>3</sup>	
Z	2	
Density (calculated)	1.385 Mg/m <sup>3</sup>	
Absorption coefficient	0.599 mm <sup>-1</sup>	
F(000)	888	
Crystal size	0.300 x 0.250 x 0.070 mm <sup>3</sup>	
Theta range for data collection	1.876 to 38.568°.	
Index ranges	-18 ≤ h ≤ 18, -31 ≤ k ≤ 31, -19 ≤ l ≤ 19	
Reflections collected	177765	
Independent reflections	23140 [R(int) = 0.0297]	
Completeness to theta = 25.242°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Refinement method	Full-matrix least-squares on F <sup>2</sup>	
Data / restraints / parameters	23140 / 1 / 475	

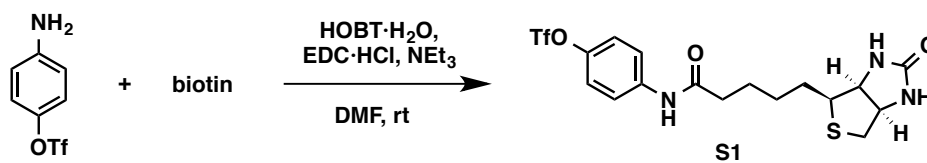
Goodness-of-fit on F <sup>2</sup>	1.023
Final R indices [I>2σ(I)]	R1 = 0.0230, wR2 = 0.0518
R indices (all data)	R1 = 0.0265, wR2 = 0.0529
Absolute structure parameter	-0.009(2)
Extinction coefficient	n/a
Largest diff. peak and hole	0.588 and -0.463 e.Å <sup>-3</sup>

### 3. Oxidative Addition Complexes

#### *Synthesis of Starting Materials*

4-Aminophenyl trifluoromethanesulfonate,<sup>5</sup> fluorescein monotriflate,<sup>6</sup> and 2-ethyl-6-methylpyridin-3-yl trifluoromethanesulfonate<sup>7</sup> were prepared according to literature procedures.

#### **Aryl trifluoromethanesulfonate S1.**



In an oven-dried round-bottom flask (250 mL) biotin (810.3 mg, 3.32 mmol) was suspended in anhydrous DMF (20 mL). Subsequently, 1-hydroxybenzotriazole hydrate (609.4 mg, 3.98 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (763.0 mg, 3.98 mmol), and NEt<sub>3</sub> (0.88 mL, 3.32 mmol) were added to the reaction and the mixture was stirred at room temperature for 15 min. After this time, 4-aminophenyl trifluoromethanesulfonate (800 mg, 3.32 mmol) was added and the reaction was stirred for an additional 3 h. Upon completion of the reaction, DMF was removed under vacuum. Addition of CH<sub>2</sub>Cl<sub>2</sub> resulted in the formation of a white precipitate, which was filtered, washed with additional CH<sub>2</sub>Cl<sub>2</sub> and pentane, and dried under reduced pressure to afford the final product as a white solid (829.6 mg, 54%).

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.01 (s, br, NH), 10.18 (s, br, NH), 7.74 (d, *J* = 8.8 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 6.43 (s, 1H), 6.36 (s, 1H), 4.31 (m, 1H), 4.13 (m, 1H), 3.10 (m, 1H), 2.82 (dd, *J* = 12.5, 4.8 Hz, 2H), 2.58 (d, *J* = 12.4 Hz, 1H), 2.33 (t, *J* = 7.5 Hz, 1H), 2.20 (t, *J* = 7.4 Hz, 1H), 1.62 (m, 2H), 1.50 (m, 2H), 1.35 (m, 2H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 174.48, 171.62, 162.77, 162.76, 144.01, 139.67, 121.86, 120.46, 118.28 (q, *J* = 321 Hz, CF<sub>3</sub>),

61.10, 61.09, 59.25, 59.23, 55.43, 55.41, 36.23, 33.52, 28.22, 28.15, 28.11, 28.08, 25.02, 24.57 (observed complexity is due to C–F coupling).  $^{19}\text{F}$  NMR (282 MHz, DMSO- $d_6$ )  $\delta$  -73.16. FT-IR (neat,  $\text{cm}^{-1}$ ): 3262.53, 2923.74, 1698.21, 1662.2, 1529.16, 1501.48, 1464.35, 1405.69, 1323.64, 1250.64, 1210.37, 1137.17, 883.25, 839.93, 694.8, 667.57, 633.6, 603.6. HRMS electrospray ( $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{17}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_5\text{S}_2$ : 467.0869, found 468.0885;  $[2\text{M} + \text{H}]^+$  calcd for  $\text{C}_{34}\text{H}_{41}\text{F}_6\text{N}_6\text{O}_{10}\text{S}_4$ : 935.1671, found 935.1766.

### **Synthesis of Oxidative Addition Complexes**

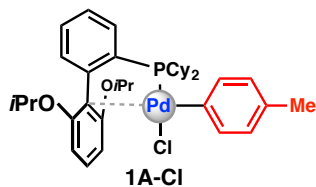
$[(1,5\text{-COD})\text{PdCl}_2]$  was prepared according to literature procedure.<sup>8,9</sup>  $[(1,5\text{-COD})\text{Pd}(\text{CH}_2\text{TMS})_2]$  was prepared according to a modified literature procedure.<sup>10</sup>

**Synthesis of  $[(1,5\text{-COD})\text{Pd}(\text{CH}_2\text{TMS})_2]$ .** A flame-dried Schlenk flask (100 mL), equipped with a magnetic stir bar, was filled with argon and charged with  $(1,5\text{-COD})\text{PdCl}_2$  (3.15g, 11.05 mmol). The flask was put under vacuum and filled back with argon. The procedure was repeated 3 consecutive times. Diethyl ether (49.3 mL) was introduced via syringe, the reaction was cooled to  $-40\text{ }^\circ\text{C}$  (acetonitrile/dry ice bath) and  $\text{TMSCH}_2\text{MgCl}$  (23.4 mL, 1.0 M) was added dropwise over 10–20 min. The reaction was stirred at  $-40\text{ }^\circ\text{C}$  for 1 h and then at  $0\text{ }^\circ\text{C}$  (ice/water bath) for an additional 20 min. Acetone (1.3 mL) was added at  $0\text{ }^\circ\text{C}$ , the reaction mixture was stirred for 5 min, after which the solvent was removed under vacuum using an external trap (the flask was kept at  $0\text{ }^\circ\text{C}$ ). The flask was then opened to air, pentane (100 mL) was added and the crude material was filtered through a pad of Celite into a new round-bottom flask (500 mL) at  $0\text{ }^\circ\text{C}$ . The filter cake was washed with pentane (50 mL  $\times$  2). Pentane from the combined washes was removed with the aid of a rotary evaporator at  $0\text{ }^\circ\text{C}$  (ice/water bath). The resulting white solid was dried under vacuum for 2 h at  $0\text{ }^\circ\text{C}$ , and transferred into a 20 mL scintillation vial in the glovebox (3.00 g, 70%). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the obtained material are identical to those reported in the literature.<sup>10</sup> The title compound was stored in the glovebox at  $-20\text{ }^\circ\text{C}$ .

### **Representative Procedure for the Synthesis of Oxidative Addition Complexes.**

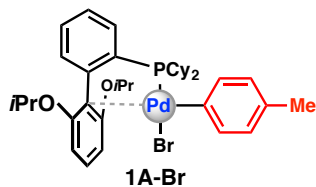
In a nitrogen-filled glovebox, an oven-dried scintillation vial (10 mL), which was equipped with a magnetic stir bar, was charged with RuPhos (1.1 equiv), Ar–X (1.1 equiv), and cyclohexane. Solid  $(\text{COD})\text{Pd}(\text{CH}_2\text{SiMe}_3)_2$  (1 equiv) was added rapidly in one portion and the resulting solution was stirred for 16 h at rt. After this time, pentane (3 mL) was added and the resulting mixture was placed into a  $-20\text{ }^\circ\text{C}$  freezer for 3 h. The vial was then taken outside of the glovebox, and the resulting precipitate was filtered, washed with pentane (3  $\times$  3 mL), and dried

under reduced pressure to afford the oxidative addition complex.



Following the general procedure, a mixture containing 4-chlorotoluene (17  $\mu$ L, 0.14 mmol), RuPhos (66 mg, 0.14 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (50 mg, 0.13 mmol) was stirred at rt in cyclohexane (1.5 mL) for 16 h. General work up afforded **1A-Cl** as a white solid (68.7 mg, 77%).

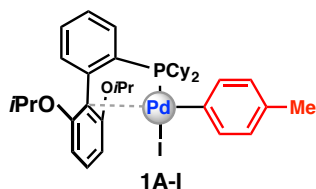
<sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$  7.62 (t, *J* = 8.4 Hz, 2H), 7.43 (tt, *J* = 7.6, 1.5 Hz, 2H), 7.38 (m, 2H), 6.93 (dd, *J* = 8.2, 2.1 Hz, 2H), 6.86 (ddd, *J* = 7.6, 3.0, 1.3 Hz, 1H), 6.77 (d, *J* = 8.1 Hz, 2H), 6.63 (d, *J* = 8.5 Hz, 2H), 4.62 (hept, *J* = 6.1 Hz, 2H), 2.21 (s, 3H), 2.12 (m, 2H), 1.75 (d, *J* = 12.1 Hz, 6H), 1.63 (m, 6H), 1.38 (d, *J* = 6.0 Hz, 6H), 1.16 (m, 6H), 1.01 (d, *J* = 6.1 Hz, 6H), 0.78 (m, 2H). <sup>13</sup>C NMR (101 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$  158.94, 145.05, 144.88, 136.63, 136.60, 134.55, 133.94, 133.75, 133.74, 133.59, 132.58, 132.48, 132.37, 131.03, 130.50, 130.48, 127.88, 127.86, 126.32, 126.26, 111.65, 111.61, 107.08, 70.83, 33.86, 33.59, 28.26, 27.77, 27.75, 27.29, 27.16, 26.99, 26.95, 26.84, 26.12, 21.95, 21.40, 20.33 (observed complexity is due to C–P coupling). <sup>31</sup>P NMR (121 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$  31.40. FT-IR (neat, cm<sup>–1</sup>): 2973.28, 2927.26, 2850.11, 1595.76, 1585.67, 1482.98, 1452.13, 1382.47, 1367.3, 1293.75, 1271.69, 1238.07, 1136.18, 1114.6, 1052.64, 1014.05, 1002.69, 948.47, 941.24, 915.67, 894.84, 888.54, 847.55, 797.78, 759.42, 745.72, 733.6, 720.03, 668.01, 610.21. Anal. Calcd. for C<sub>37</sub>H<sub>50</sub>ClO<sub>2</sub>PPd: C, 63.52; H, 7.20. Found: C, 63.47; H, 7.24.



Following the general procedure, a mixture containing 4-bromotoluene (24.2 mg, 0.14 mmol), RuPhos (66.0 mg, 0.14 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (50.0 mg, 0.13 mmol) was stirred at rt in cyclohexane (1 mL) for 16 h. General work up afforded **1A-Br** as an off-white solid (78.4 mg, 82%).

<sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$  7.61 (m, 2H), 7.43 (tt, *J* = 7.5, 1.6 Hz, 1H), 7.37 (m, 1H), 6.91

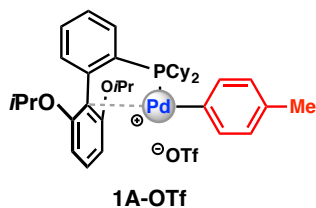
(dd,  $J = 8.2, 2.3$  Hz, 2H), 6.86 (ddd,  $J = 7.8, 3.1, 1.5$  Hz, 1H), 6.76 (d,  $J = 8.0$  Hz, 2H), 6.64 (d,  $J = 8.4$  Hz, 2H), 4.60 (hept,  $J = 6.1$  Hz, 2H), 2.22 (s, 3H), 2.14 (m, 2H), 1.77 (m, 6H), 1.60 (m, 6H), 1.38 (d,  $J = 6.0$  Hz, 6H), 1.17 (m, 6H), 1.01 (d,  $J = 6.0$  Hz, 6H), 0.78 (m, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  159.42, 145.38, 145.20, 137.74, 137.70, 134.88, 134.18, 133.84, 133.11, 133.01, 132.94, 131.62, 131.56, 130.99, 130.97, 128.20, 126.81, 126.76, 112.44, 112.41, 107.88, 71.44, 34.40, 34.14, 28.73, 28.17, 28.15, 27.82, 27.69, 27.49, 27.46, 27.35, 26.60, 22.46, 21.93, 20.79 (observed complexity is due to C–P coupling).  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  29.89. FT-IR (neat,  $\text{cm}^{-1}$ ): 2980.24, 2917.56, 2844.37, 1587.1, 1478.11, 1464.73, 1451.26, 1442.93, 1384.38, 1372.21, 1349.62, 1333.2, 1289.54, 1255.37, 1207.89, 1112.89, 1069.63, 1054.03, 1010.41, 902.77, 892.23, 851.92, 837.45, 791.2, 785.02, 767.28, 760.24, 747.31, 736.44, 700.12, 675.71, 612.24. HRMS electrospray ( $m/z$ ):  $[\text{M} - \text{Br}]^+$  calcd for  $\text{C}_{37}\text{H}_{50}\text{O}_2\text{PPd}$ : 663.2596, found 663.2603.



Following the general procedure, a mixture containing 4-iodotoluene (61.7 mg, 0.28 mmol), RuPhos (131.9 mg, 0.28 mmol), and (COD)Pd( $\text{CH}_2\text{SiMe}_3$ )<sub>2</sub> (100.0 mg, 0.26 mmol) was stirred at rt in cyclohexane (1.5 mL) for 16 h. General work up afforded **1A-I** as a bright yellow solid (180.0 mg, 89%).

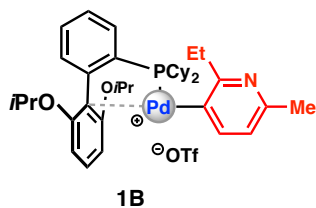
$^1\text{H}$  NMR (400 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  7.60 (m, 2H), 7.42 (m, 1H), 7.36 (m, 1H), 6.89 (dd,  $J = 8.2, 2.0$  Hz, 2H), 6.84 (ddd,  $J = 7.5, 2.9, 1.2$  Hz, 2H), 6.74 (d,  $J = 8.0$  Hz, 2H), 6.66 (d,  $J = 8.4$  Hz, 2H), 4.59 (hept,  $J = 6.2$  Hz, 2H), 2.22 (s, 3H), 2.14 (m, 2H), 1.77 (m, 6H), 1.59 (m, 6H), 1.38 (d,  $J = 6.0$  Hz, 6H), 1.18 (m, 6H), 1.01 (d,  $J = 6.1$  Hz, 6H), 0.77 (m, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  159.56, 145.15, 144.97, 138.88, 138.85, 134.81, 133.96, 133.64, 133.19, 133.08, 132.67, 131.63, 130.96, 130.94, 127.85, 127.84, 126.76, 126.71, 126.62, 112.67, 112.63, 108.32, 71.59, 34.38, 34.13, 28.63, 28.03, 28.00, 27.87, 27.74, 27.48, 27.37, 26.57, 26.55, 22.48, 21.97, 20.71 (observed complexity is due to C–P coupling).  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  25.64. FT-IR (neat,  $\text{cm}^{-1}$ ): 2975.8, 2922.64, 2844.86, 1585.12, 1569.19, 1477.72, 1454.19, 1386.62, 1374.01, 1326.62, 1282, 1251.88, 1207.49, 1171.57, 1135.22, 1112.08, 1065.38, 1055.2, 1010.27, 997.53, 846.52, 787.24, 772.21, 767.54, 759.04, 747.91, 734.94, 727.13, 696.29, 676.75, 637.81, 612.42,

602.4, 579.03. Anal. Calcd. for C<sub>37</sub>H<sub>50</sub>IO<sub>2</sub>PPd: C, 56.18; H, 6.37. Found: C, 55.69; H, 6.42.



Following the general procedure, a mixture containing 4-tolyl trifluoromethanesulfonate (100.0 mg, 0.42 mmol), RuPhos (194.0 mg, 0.42 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (147.0 mg, 0.38 mmol) was stirred at rt in cyclohexane (1.5 mL) for 16 h. General work up afforded **1A-OTf** as an off-white solid (270.0 mg, 88%).

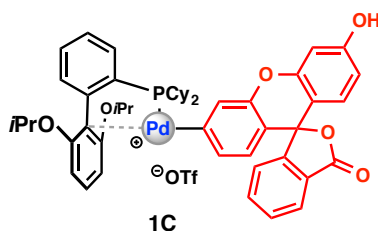
<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN) δ 7.73 (t, *J* = 7.7 Hz, 1H), 7.64 (t, *J* = 8.5 Hz, 1H), 7.52 (m, 1H), 7.47 (m, 1H), 6.93 (m, 4H), 6.77 (m, 3H), 4.68 (hept, *J* = 6.2 Hz, 2H), 2.22 (m, 5H), 1.79 (m, 6H), 1.60 (m, 6H), 1.41 (d, *J* = 6.0 Hz, 6H), 1.20 (m, 6H), 1.03 (d, *J* = 6.0 Hz, 6H), 0.69 (m, 2H). <sup>13</sup>C NMR (101 MHz, CD<sub>3</sub>CN) δ 163.57, 145.97, 145.80, 138.39, 137.14, 137.10, 136.03, 134.17, 133.30, 133.28, 133.06, 133.01, 132.89, 130.30, 130.27, 128.37, 128.30, 107.26, 72.63, 35.36, 35.05, 29.55, 29.44, 28.02, 27.95, 27.82, 27.75, 27.63, 27.09, 27.07, 22.58, 22.30, 21.04 (observed complexity is due to *C-P* coupling). <sup>31</sup>P NMR (121 MHz, CD<sub>3</sub>CN) δ 41.32. <sup>19</sup>F NMR (376 MHz, CD<sub>3</sub>CN) δ -82.72. FT-IR (neat, cm<sup>-1</sup>): 2973.6, 2921.73, 2850.32, 1589.06, 1480.22, 1451.38, 1389, 1378.25, 1308.92, 1255.2, 1229.22, 1208.11, 1177.28, 1141.2, 1110.23, 1066.14, 1055.85, 1016.65, 900.16, 849.85, 791.27, 767.96, 736.56, 628.06, 613.09. Anal. Calcd. for C<sub>38</sub>H<sub>50</sub>F<sub>3</sub>O<sub>5</sub>PPdS: C, 56.12; H, 6.20. Found: C, 56.39; H, 6.46.



Following the general procedure, a mixture containing 2-ethyl-6-methylpyridin-3-yl trifluoromethanesulfonate (76.0 mg, 0.28 mmol, *Note*: 2.2 equiv was used), RuPhos (66.0 mg, 0.141 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (50.0 mg, 0.129 mmol) was stirred at rt in cyclohexane (0.75 mL) for 16 h. General work up afforded **1B** as a light yellow solid (95.0 mg, 88%).

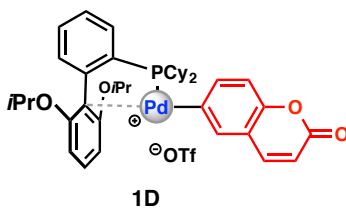
<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN) δ 7.70 (m, 2H), 7.53 (tt, *J* = 7.6, 1.6 Hz, 1H), 7.47 (tt, *J* = 7.4, 1.6 Hz, 1H), 6.99 (dd, *J* = 7.9, 2.8 Hz, 1H), 6.79 (m, 4H), 4.71 (m, 1H), 4.63 (m, 1H), 3.17 (m, 2H),

2.40 (m, 5H), 2.12 (m, 2H), 1.76 (m, 6H), 1.49 (m, 5H), 1.40 (d,  $J = 6.0$  Hz, 3H), 1.34 (d,  $J = 6.0$  Hz, 3H), 1.25 (m, 5H), 1.13 (m, 9H), 0.92 (d,  $J = 6.0$  Hz, 3H), 0.17 (m, 1H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{CN}$ )  $\delta$  164.70, 164.45, 164.09, 154.73, 145.65, 145.48, 143.59, 143.54, 138.99, 135.58, 135.14, 133.08, 133.06, 132.54, 132.42, 132.31, 130.75, 128.13, 128.07, 122.77, 106.93, 106.75, 73.27, 72.21, 36.84, 35.72, 35.42, 33.66, 33.37, 31.96, 31.93, 29.44, 27.82, 27.75, 27.66, 27.60, 27.57, 27.45, 27.11, 26.99, 26.88, 26.83, 26.67, 26.61, 23.36, 22.37, 22.24, 22.09, 21.80, 13.29 (observed complexity is due to  $C-P$  coupling).  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_3\text{CN}$ )  $\delta$  41.30.  $^{19}\text{F}$  NMR (376 MHz,  $\text{CD}_3\text{CN}$ )  $\delta$  -78.65. FT-IR (neat,  $\text{cm}^{-1}$ ): 2926.73, 2852.81, 1589.56, 1558.05, 1447.32, 1385.47, 1307.23, 1274.47, 1252.38, 1225.28, 1200.75, 1173.43, 1143.04, 1104.51, 1061.88, 1030.39, 1010.98, 892.25, 849.62, 820.09, 806.38, 769.71, 748.12, 737.28, 627.7, 609.26. Anal. Calcd. for  $\text{C}_{39}\text{H}_{53}\text{F}_3\text{NO}_5\text{PPdS}$ : C, 55.61; H, 6.34. Found: C, 55.15; H, 6.19.



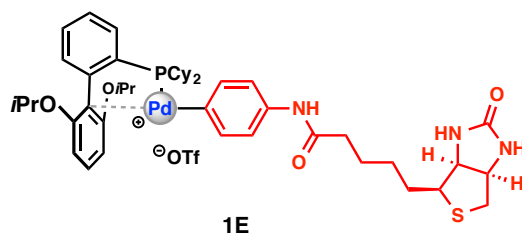
Following the general procedure, a mixture containing fluorescein monotrifluoromethanesulfonate (52.5 mg, 0.11 mmol, *Note*: used as the limiting reagent), RuPhos (66.0 mg, 0.14 mmol), and (COD)Pd( $\text{CH}_2\text{SiMe}_3$ )<sub>2</sub> (50.0 mg, 0.13 mmol) was stirred in THF (0.75 mL) at rt for 16 h using aluminum foil for light exclusion. General work up afforded **1C** as a bright orange precipitate (107.5 mg, 92%).

$^1\text{H}$  NMR (400 MHz, DMSO) *Complex spectrum observed (see the end of Supporting Information)*.  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  44.41.  $^{19}\text{F}$  NMR (376 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  -79.08. FT-IR (neat,  $\text{cm}^{-1}$ ): 2929.22, 2851.87, 1761.86, 1625.02, 1587.77, 1564.66, 1509.79, 1443.16, 1384.65, 1346.61, 1274.48, 1252.52, 1222.15, 1154.31, 1106.89, 1083.41, 1064.08, 1029.68, 981.25, 949.65, 934.76, 891.29, 849.46, 820.67, 763.46, 695.31, 660.91, 636.48, 626.65. Anal. Calcd. for  $\text{C}_{51}\text{H}_{54}\text{F}_3\text{O}_9\text{PPdS}$ : C, 59.05; H, 5.25. Found: C, 58.87; H, 5.42.



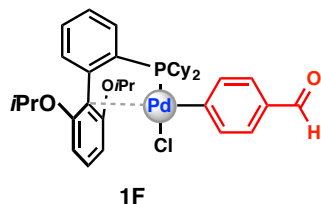
Following the general procedure, a mixture containing 2-oxo-2*H*-chromen-6-yl trifluoromethanesulfonate (38.2 mg, 0.13 mmol, *Note*: 1.01 equiv was used), RuPhos (66.0 mg, 0.14 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (50.0 mg, 0.13 mmol) was stirred at rt in THF (0.75 mL) for 16 h. General work up afforded **1D** as a light yellow solid (103.3 mg, 93%).

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN) δ 7.73 (d, *J* = 8.0 Hz, 1H), 7.58 (m, 3H), 7.45 (m, 1H), 7.29 (d, *J* = 8.5 Hz, 1H), 7.14 (s, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 6.88 (m, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 6.33 (d, *J* = 9.5 Hz, 1H), 4.69 (hept, *J* = 6.1 Hz, 2H), 2.10 (s, br, 2H), 1.81 (m, 6H), 1.64 (m, 6H), 1.39 (d, *J* = 6.0 Hz, 6H), 1.28 (m, 2H), 1.13 (m, 4H), 1.05 (d, *J* = 6.1 Hz, 6H), 0.84 (m, 2H). <sup>13</sup>C NMR (101 MHz, CD<sub>3</sub>CN) δ 161.41, 153.57, 144.67, 139.93, 139.90, 135.33, 135.29, 132.85, 128.01, 127.95, 117.07, 116.41, 107.42, 107.38, 72.69, 35.73 (br), 29.66, 27.66, 27.61, 27.53, 27.41, 27.24, 26.73, 26.72, 22.14 (observed complexity is due to C–P coupling). <sup>31</sup>P NMR (121 MHz, CD<sub>3</sub>CN) δ 42.43. <sup>19</sup>F NMR (376 MHz, CD<sub>3</sub>CN) δ -78.89. FT-IR (neat, cm<sup>-1</sup>): 2979.13, 2924.12, 2848.66, 1721.51, 1584.47, 1543.58, 1453.67, 1413.89, 1384.87, 1315.94, 1254.76, 1227.39, 1213.36, 1195.09, 1159.85, 1139.1, 1112.08, 1071.78, 1030.22, 1004.07, 891.57, 877.55, 851.16, 826.12, 772.82, 758.61, 747.4, 737.17, 632.64. Anal. Calcd. for C<sub>40</sub>H<sub>48</sub>F<sub>3</sub>O<sub>7</sub>PPdS: C, 55.40; H, 5.58. Found: C, 55.77; H, 5.49.



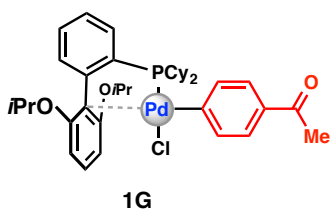
Following the general procedure, a mixture containing aryl trifluoromethanesulfonate **S1** (100.0 mg, 0.21 mmol, *Note*: 1 equiv was used), RuPhos (109.8 mg, 0.24 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (83.2 mg, 0.21 mmol) was stirred in THF (1.5 mL) at rt for 16 h. General work up afforded **1E** as a light orange solid (179.0 mg, 80%).

<sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) *Complex spectrum obsesrved (see the end of Supporting Information)*. <sup>31</sup>P NMR (121 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 42.30. <sup>19</sup>F NMR (376 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ -78.99. FT-IR (neat, cm<sup>-1</sup>): 3301.3 (br), 2929.22, 2853.24, 1699.37, 1579.49, 1519.47, 1480.55, 1449.2, 1383.79, 1330.61, 1250.31, 1154.46, 1106.11, 1063.64, 1029.55, 1004.78, 898.47, 849.61, 819.71, 763.79, 736.07, 667.69, 636.99. HRMS electrospray (*m/z*): [M – OSO<sub>2</sub>CF<sub>3</sub>]<sup>+</sup> calcd for C<sub>46</sub>H<sub>47</sub>N<sub>3</sub>O<sub>4</sub>PPdS<sub>2</sub>: 890.3329, found 890.3325.



Following the general procedure, a mixture containing 4-chlorobenzaldehyde (39.7 mg, 0.28 mmol), RuPhos (131.9 mg, 0.28 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (100.0 mg, 0.26 mmol) was stirred in cyclohexane (1.5 mL) at rt for 16 h. General work up afforded **1F** as a white solid (166.0 mg, 91%).

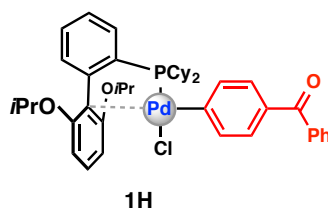
<sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 9.83 (s, 1H), 7.64 (m, 2H), 7.46 (tt, *J* = 7.7, 1.6 Hz, 1H), 7.40 (m, 5H), 6.89 (ddd, *J* = 7.7, 3.2, 1.4 Hz, 1H), 6.66 (d, *J* = 8.5 Hz, 2H), 4.64 (hept, *J* = 6.1 Hz, 2H), 2.11 (m, 2H), 1.68 (m, 12H), 1.39 (d, *J* = 6.0 Hz, 6H), 1.18 (m, 6H), 1.03 (d, *J* = 6.1 Hz, 6H), 0.77 (m, 2H). <sup>13</sup>C NMR (101 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 192.86, 159.84, 154.40, 145.37, 145.19, 138.25, 138.21, 135.60, 133.61, 133.26, 133.10, 132.99, 131.50, 131.32, 131.30, 127.36, 127.06, 127.00, 111.36, 111.32, 107.76, 71.62, 34.46, 34.19, 28.82, 28.37, 28.35, 27.68, 27.54, 27.48, 27.35, 27.23, 26.59, 26.58, 22.44, 21.86 (observed complexity is due to C–P coupling). <sup>31</sup>P NMR (121 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 33.19. FT-IR (neat, cm<sup>-1</sup>): 2928.23, 2849.21, 2812.38, 2721.21, 1693.19, 1652.71, 1591.79, 1572.27, 1551.15, 1506.8, 1456.37, 1382.73, 1371.34, 1329.84, 1295.43, 1268.02, 1243.71, 1216.23, 1166.08, 1113.54, 1074.07, 1054.93, 1011.02, 1004.34, 939.3, 917.45, 896.81, 888.89, 848.95, 837.59, 810.58, 778.57, 763.38, 744.18, 737.98, 724, 674.14, 612.18. Anal. Calcd. for C<sub>37</sub>H<sub>48</sub>ClO<sub>3</sub>PPd: C, 62.27; H, 6.78. Found: C, 62.45; H, 6.93.



Following the general procedure, a mixture containing 4-chloroacetophenone (36.7 μL, 0.28 mmol), RuPhos (131.9 mg, 0.28 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (100.0 mg, 0.26 mmol) was stirred in cyclohexane (1.5 mL) at rt for 16 h. General work up afforded **1G** as a white solid (187.1 mg, 80%).

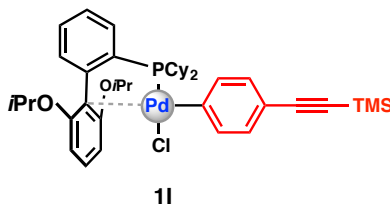
<sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 7.63 (m, 2H), 7.47 (m, 3H), 7.40 (m, 1H), 7.27 (m, 2H), 6.88 (ddd, *J* = 7.7, 3.2, 1.4 Hz, 1H), 6.65 (d, *J* = 8.5 Hz, 2H), 4.64 (hept, *J* = 6.1 Hz, 2H), 2.49 (s, 3H),

2.12 (m, 2H), 1.68 (m, 12H), 1.39 (d,  $J = 6.0$  Hz, 6H), 1.18 (m, 6H), 1.02 (d,  $J = 6.1$  Hz, 6H), 0.79 (m, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  198.60, 159.72, 151.23, 151.22, 145.37, 145.20, 137.70, 137.67, 135.45, 133.70, 133.58, 133.33, 133.09, 132.99, 131.51, 131.25, 131.23, 127.01, 126.95, 126.31, 126.30, 111.61, 111.58, 107.76, 71.59, 34.45, 34.18, 28.83, 28.38, 28.36, 27.68, 27.55, 27.49, 27.36, 27.24, 26.74, 26.61, 26.60, 22.45, 21.86 (observed complexity is due to  $C-P$  coupling).  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  33.19. FT-IR (neat,  $\text{cm}^{-1}$ ): 2937.05, 2849.21, 1672.81, 1590.71, 1570.43, 1541.83, 1453.46, 1383.73, 1370.46, 1350.76, 1296.23, 1270.06, 1246.23, 1177.32, 1131.31, 1112.59, 1075.99, 1057.28, 1008.91, 952, 919.43, 900.05, 847.69, 820.15, 807.81, 777.15, 758.83, 739.52, 724.42, 667.86, 614.63, 596.58, 578.79, 572.06, 557.85, 555.93, 553.94. Anal. Calcd. for  $\text{C}_{38}\text{H}_{50}\text{ClO}_3\text{PPd}$ : C, 62.72; H, 6.93. Found: C, 62.68; H, 6.98.



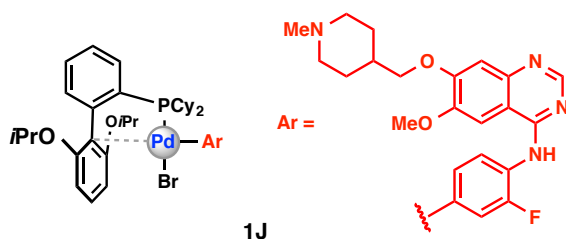
Following the general procedure, a mixture containing 4-chlorobenzophenone (61.2 mg, 0.28 mmol), RuPhos (131.9 mg, 0.28 mmol), and  $(\text{COD})\text{Pd}(\text{CH}_2\text{SiMe}_3)_2$  (100.0 mg, 0.26 mmol) was stirred in cyclohexane (1.5 mL) at rt for 16 h. General work up afforded **1H** as a white solid (170.3 mg, 84%).

$^1\text{H}$  NMR (400 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  7.72 (m, 2H), 7.65 (m, 2H), 7.56 (m, 1H), 7.46 (m, 3H), 7.39 (m, 3H), 7.31 (dd,  $J = 8.5, 2.0$  Hz, 2H), 6.89 (ddd,  $J = 7.6, 3.1, 1.3$  Hz, 1H), 6.66 (d,  $J = 8.4$  Hz, 2H), 4.64 (hept,  $J = 6.2$  Hz, 2H), 2.15 (m, 2H), 1.68 (m, 12H), 1.39 (d,  $J = 6.0$  Hz, 6H), 1.18 (m, 6H), 1.03 (d,  $J = 6.0$  Hz, 6H), 0.79 (m, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  197.01, 159.80, 150.68, 145.42, 145.25, 139.09, 137.53, 137.49, 135.53, 133.75, 133.38, 133.30, 133.09, 132.98, 132.21, 131.53, 131.28, 131.26, 130.25, 128.58, 128.33, 128.31, 127.02, 126.96, 111.48, 111.44, 107.73, 71.57, 34.40, 34.13, 28.79, 28.35, 28.33, 27.72, 27.59, 27.36, 27.25, 26.58, 22.44, 21.88 (observed complexity is due to  $C-P$  coupling).  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  33.29. FT-IR (neat,  $\text{cm}^{-1}$ ): 2974.7, 2926.53, 2849.69, 1652.22, 1567.46, 1538.81, 1456.24, 1382.54, 1371.38, 1314.3, 1300.72, 1278.58, 1244.96, 1174.95, 1109.61, 1052.35, 1011.48, 1000.34, 935.09, 919.51, 849.46, 832.32, 785.17, 758.91, 735.49, 725.02, 699.26, 668.36, 651.79, 612.29. Anal. Calcd. for  $\text{C}_{43}\text{H}_{52}\text{ClO}_3\text{PPd}$ : C, 65.40; H, 6.64. Found: C, 65.62; H, 6.38.



Following the general procedure, a mixture containing (4-chlorophenylethynyl)trimethylsilane (71.6 mg, 0.34 mmol), RuPhos (131.9 mg, 0.28 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (100.0 mg, 0.26 mmol) was stirred in cyclohexane (1.5 mL) at rt for 16 h. General work up afforded **1I** as a white solid (157.7 mg, 78%).

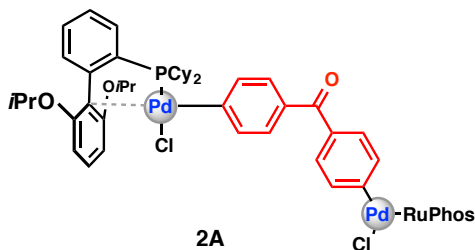
<sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 7.62 (m, 2H), 7.45 (m, 1H), 7.39 (m, 1H), 7.08 (dd, *J* = 8.4, 2.0 Hz, 2H), 7.01 (d, *J* = 8.1 Hz, 2H), 6.88 (ddd, *J* = 7.6, 3.1, 1.4 Hz, 1H), 6.65 (d, *J* = 8.5 Hz, 2H), 4.63 (hept, *J* = 6.1 Hz, 2H), 2.21 (m, 2H), 1.67 (m, 13H), 1.38 (d, *J* = 6.0 Hz, 6H), 1.17 (m, 6H), 1.01 (d, *J* = 6.1 Hz, 6H), 0.82 (m, 2H), 0.22 (s, 9H). <sup>13</sup>C NMR (101 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 159.52, 145.34, 145.17, 142.81, 137.56, 137.52, 135.24, 133.74, 133.37, 133.11, 133.00, 131.50, 131.14, 130.13, 126.97, 126.91, 118.26, 111.99, 111.96, 107.81, 106.45, 92.74, 71.56, 34.42, 34.15, 28.87, 28.34, 27.72, 27.59, 27.49, 27.39, 27.28, 26.64, 22.46, 21.84, 0.30 (observed complexity is due to C–P coupling). <sup>31</sup>P NMR (121 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 32.69. FT-IR (neat, cm<sup>-1</sup>): 2920.64, 2850.24, 2153.99, 1573.62, 1475.48, 1452.03, 1383.03, 1367.97, 1294.31, 1273.73, 1238.97, 1175.48, 1110.6, 1054.15, 1012.36, 1003.56, 941.96, 916.46, 866.35, 839.95, 815.21, 787.82, 754.48, 745.71, 730.56, 720.42, 698.18, 659.3. Anal. Calcd. for C<sub>41</sub>H<sub>56</sub>ClO<sub>2</sub>PPdSi: C, 62.99; H, 7.22. Found: C, 63.25; H, 7.09.



Following the general procedure, a mixture containing Vandetanib (61.7 mg, 0.13 mmol, *Note*: 1.01 equiv was used), RuPhos (66.0 mg, 0.14 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (50.0 mg, 0.13 mmol) was stirred in THF (1.5 mL) at rt for 16 h. General work up afforded **1J** as an off-white solid (119.0 mg, 88%).

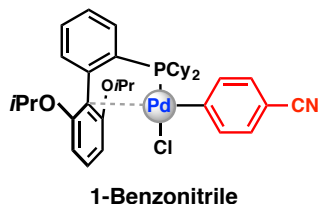
<sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 8.54 (s, 1H), 7.85 (t, *J* = 8.6 Hz, 1H), 7.65 (m, 2H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.4 Hz, 1H), 7.20 (s, 1H), 7.11 (m, 1H), 7.04 (s, 1H), 6.93 (m, 2H), 6.88

(dd,  $J = 7.6, 3.2$  Hz, 1H), 6.67 (d,  $J = 8.5$  Hz, 2H), 4.63 (hept,  $J = 6.0$  Hz, 2H), 3.98 (s, 3H), 2.85 (m, 2H), 2.23 (m, 5H), 1.85 (m, 17H), 1.40 (m, 8H), 1.22 (m, 8H), 1.03 (d,  $J = 6.1$  Hz, 6H), 0.90 (m, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  159.92, 156.93, 154.90, 154.48, 153.96, 150.35, 147.98, 145.33, 145.16, 135.49, 133.80, 133.52, 133.45, 133.14, 133.03, 132.04, 131.99, 131.57, 131.27, 131.25, 126.97, 126.92, 124.08, 123.88, 122.96, 122.85, 122.49, 111.38, 111.34, 109.50, 109.05, 107.96, 100.30, 74.14, 71.64, 56.91, 55.89, 46.82, 35.58, 34.50, 34.23, 29.68, 28.77, 28.34, 27.80, 27.67, 27.39, 27.28, 26.58, 22.43, 22.39, 21.91 (observed complexity is due to  $\text{C-P}$  coupling).  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  32.24. FT-IR (neat,  $\text{cm}^{-1}$ ): 2926.73, 2849, 2780.97, 1619.76, 1584.47, 1495.57, 1452.88, 1416.66, 1381.79, 1333.79, 1248.7, 1209.65, 1138.06, 1110.84, 1061.64, 1000.91, 940.37, 884.58, 849.88, 817.62, 762.84, 737.25, 667.56, 630.8. Anal. Calcd. for  $\text{C}_{52}\text{H}_{67}\text{BrFN}_4\text{O}_4\text{PPd}$ : C, 59.57; H, 6.44. Found: C, 59.24; H, 6.41.



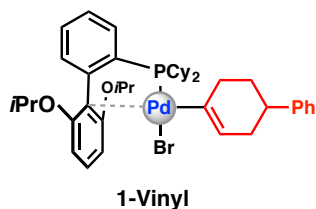
Following a slightly modified general procedure, a mixture of 4,4'-dichlorobenzophenone (30.0 mg, 0.12 mmol, 1 equiv), RuPhos (139.4 mg, 0.30 mmol, 2.5 equiv), and  $(\text{COD})\text{Pd}(\text{CH}_2\text{SiMe}_3)_2$  (116.2 mg, 0.30 mmol, 2.5 equiv) was stirred in cyclohexane (1.2 mL) at rt for 16 h. General work up afforded **2A** as a beige solid (146.8 mg, 88%).

$^1\text{H}$  NMR (400 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  7.64 (m, 4H), 7.45 (m, 2H), 7.39 (m, 2H), 7.32 (d,  $J = 8.0$  Hz, 4H), 7.25 (dd,  $J = 8.4, 2.1$  Hz, 4H), 6.88 (ddd,  $J = 7.7, 3.1, 1.3$  Hz, 2H), 6.65 (d,  $J = 8.5$  Hz, 4H), 4.64 (hept,  $J = 6.1$  Hz, 4H), 2.14 (m, 4H), 1.70 (m, 24H), 1.39 (d,  $J = 6.0$  Hz, 12H), 1.20 (m, 12H), 1.02 (d,  $J = 6.0$  Hz, 12H), 0.75 (m, 4H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  197.01, 159.78, 149.09, 145.47, 145.30, 137.25, 137.21, 135.49, 134.06, 133.94, 133.58, 133.06, 132.95, 131.55, 131.23, 131.21, 128.34, 126.98, 126.92, 111.50, 107.69, 71.53, 34.39, 34.12, 28.78, 28.32, 27.73, 27.59, 27.38, 27.27, 26.59, 22.44, 21.89 (observed complexity is due to  $\text{C-P}$  coupling).  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  33.27. FT-IR (neat,  $\text{cm}^{-1}$ ): 2924.96, 2849.75, 1652.54, 1645.7, 1569.12, 1538.83, 1456.32, 1382.56, 1371.3, 1278.13, 1244.17, 1173.52, 1110.74, 1051.09, 1010.83, 921.17, 848.83, 814.88, 784.1, 751.87, 667.12, 623.81, 612.26, 579.08. Anal. Calcd. for  $\text{C}_{73}\text{H}_{94}\text{Cl}_2\text{O}_5\text{P}_2\text{Pd}_2$ : C, 62.75; H, 6.78. Found: C, 62.36; H, 6.83.



Following the general procedure, a mixture of 4-chlorobenzonitrile (42.4 mg, 0.31 mmol), RuPhos (144.0 mg, 0.31 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (100.0 mg, 0.26 mmol) was stirred in cyclohexane (1.5 mL) at rt for 16 h. General work up afforded **1-Benzonitrile** as a white solid (186.4 mg, 99%).

<sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 7.62 (m, 2H), 7.51 (tt, *J* = 7.5, 1.6 Hz, 1H), 7.44 (m, 1H), 7.27 (dd, *J* = 8.0, 2.0 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 6.93 (dd, *J* = 7.8, 3.1 Hz, 1H), 6.70 (d, *J* = 8.4 Hz, 2H), 4.65 (hept, *J* = 6.1 Hz, 2H), 2.08 (m, 2H), 1.89 (m, 2H), 1.78 (m, 4H), 1.67 (m, 4H), 1.56 (m, 2H), 1.37 (d, *J* = 6.0 Hz, 6H), 1.20 (m, 6H), 1.03 (d, *J* = 6.0 Hz, 6H), 0.85 (m, 2H). <sup>13</sup>C NMR (101 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 160.15, 139.17, 139.13, 133.70, 133.60, 132.71, 131.75, 129.68, 127.56, 127.50, 120.54, 108.36, 107.04, 72.10, 35.11, 34.84, 29.69, 29.16, 27.81, 27.69, 27.61, 27.47, 26.85, 26.84, 22.49, 21.94. (observed complexity is due to C–P coupling). <sup>31</sup>P NMR (121 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 33.46. FT-IR (neat, cm<sup>-1</sup>): 2927.23, 2849.55, 2223.84, 1591.35, 1575.29, 1473.98, 1450.97, 1383.19, 1371.34, 1274.04, 1243.98, 1174.33, 1130.21, 1114.05, 1055.96, 1014.33, 1003.94, 849.24, 834.6, 809.39, 778.82, 761.92, 743.75, 737.28, 723.9, 714.69, 612.21. HRMS electrospray (*m/z*): [M – Cl]<sup>+</sup> calcd for C<sub>37</sub>H<sub>47</sub>NO<sub>2</sub>PPd: 674.2392, found 674.2411.



Following the general procedure, a mixture containing 4-bromo-1,2,3,6-tetrahydro-1,1'-biphenyl (30.0 mg, 0.127 mmol), RuPhos (59.0 mg, 0.127 mmol), and (COD)Pd(CH<sub>2</sub>SiMe<sub>3</sub>)<sub>2</sub> (44.7 mg, 0.115 mmol) was stirred in cyclohexane (0.75 mL) at rt for 16 h. General work up afforded **1-Vinyl** as a yellow solid (80.0 mg, 86%).

<sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 7.66 (td, *J* = 7.0, 1.9 Hz, 1H), 7.56 (t, *J* = 8.4 Hz, 1H), 7.40 (m, 2H), 7.23 (m, 4H), 7.14 (m, 1H), 6.82 (m, 1H), 6.60 (m, 2H), 4.94 (s, 1H), 4.56 (m, 2H), 2.90 (m, 3H), 1.90 (m, 18H), 1.25 (m, 22H). <sup>13</sup>C NMR: *could not be obtained due to complex decomposition in solution (CD<sub>2</sub>Cl<sub>2</sub>; > 4 h)*. <sup>31</sup>P NMR (121 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 29.17, 28.89. FT-IR

(neat,  $\text{cm}^{-1}$ ): 2970.4, 2923.13, 2844.48, 1576.18, 1455.04, 1387.01, 1375.4, 1283.43, 1252.95, 1201.89, 1172.85, 1141.69, 1110.95, 1065.66, 1026.88, 997.97, 968.22, 902.49, 891.58, 847.07, 775.05, 767.23, 759.11, 748.35, 741.77, 726.46, 694.67, 676.18, 612.58. Anal. Calcd. for  $\text{C}_{42}\text{H}_{56}\text{BrO}_2\text{PPd}$ : C, 62.26; H, 6.97. Found: C, 62.03; H, 6.85.

## 4. Peptide Experiments

### Peptide Synthesis

All peptides were synthesized on a 0.2 mmol scale using manual Fmoc-SPPS chemistry under flow using a 3 min cycle for each amino acid.<sup>11</sup> Specifically, all reagents and solvents were delivered to a stainless steel reactor containing resins at a constant flow rate using HPLC pump; the temperature of the reactor was maintained at 60 °C during the synthesis using a water bath. The procedure for each amino acid coupling cycle included: 1) a 30 s coupling with 1 mmol of the corresponding Fmoc-protected amino acid, 1 mmol HBTU, and 500  $\mu\text{L}$  of diisopropyl ethyl amine (DIPEA) in 2.5 mL of DMF at a flow rate of 6 mL/min (note that for the coupling of cysteine and tryptophan, 190  $\mu\text{L}$  of DIPEA was used to prevent racemization); 2) 1 min wash with DMF at a flow rate of 20 mL/min; 3) 20 s deprotection with 50% (v/v) piperidine in DMF at a flow rate of 20 mL/min; and 4) 1 min wash with DMF at a flow rate of 20 mL/min. After completion of the stepwise SPPS, the resin was washed thoroughly with DCM and dried under vacuum. The peptide was simultaneously cleaved from the resin and deprotected on the side-chains by treatment with 2.5% (v/v) water, 2.5% (v/v) 1,2-ethanedithiol (EDT), and 1% (v/v) triisopropylsilane in neat trifluoroacetic acid (TFA) for 2 h at room temperature. The solvent from the resulting solution containing the target peptide was evaporated by purging with nitrogen gas for 15 min. The residue was then triturated and washed with cold diethyl ether three times. The obtained gummy-like solid was dissolved in 50%  $\text{H}_2\text{O}$  : 50% acetonitrile containing 0.1% TFA and lyophilized.

### Peptide Purification

The crude peptide was dissolved in 95% A : 5% B with 6 M guanidinium hydrochloride and purified by semi-preparative RP-HPLC (Agilent Zorbax SB  $\text{C}_{18}$  column: 21.2 x 250 mm, 7  $\mu\text{m}$ , linear gradient: 5-50% B over 90 min, flow rate: 5 mL/min). Each HPLC fraction was analyzed by MALDI-TOF mass-spectrometry. Specifically, 1  $\mu\text{L}$  of each HPLC fraction was mixed with 1  $\mu\text{L}$  of  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) matrix in 75% A : 25% B and the resulting mixture was analyzed for the desired molecular mass using MALDI-TOF. The purity of the

fractions containing the desired peptide was further analyzed by analytical RP-HPLC (Agilent Zorbax SB C<sub>3</sub> column: 2.1 x 150 mm, 5 μm, gradient: 0-2 min 5% B, 2-11 min 5-65% B, 11-12 min 65% B, flow rate: 0.8 mL/min). HPLC fractions containing pure product were further confirmed by LC-MS, combined, and lyophilized. Peptides synthesized using fast flow-based SPPS and purified by RP-HPLC are listed in Table S2.

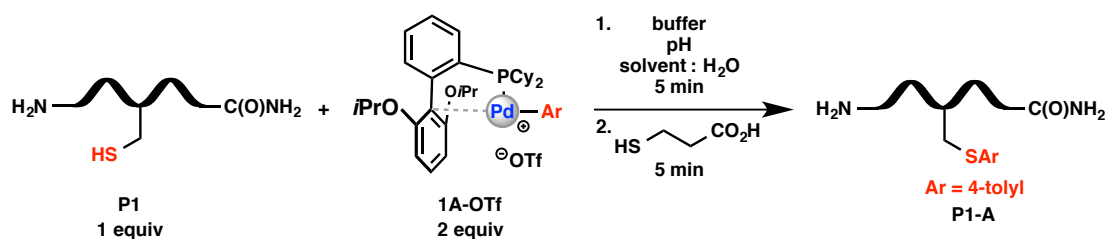
**Table S2. Sequences and masses of peptides synthesized by fast-flow peptide synthesizer.**

Peptide	Sequence <sup>a</sup>	Calculated mass	Observed mass
<b>P1</b>	NH <sub>2</sub> -RSNFYLG <b>C</b> AGLAHDKAT-CONH <sub>2</sub>	1821.89	1821.89
<b>P1-Ser</b>	NH <sub>2</sub> -RSNFYLG <b>S</b> AGLAHDKAT-CONH <sub>2</sub>	1805.92	1805.92
<b>P2</b>	NH <sub>2</sub> -RSNFFLG <b>C</b> AGA-CONH <sub>2</sub>	1140.55	1140.55
<b>P3</b>	NH <sub>2</sub> -IKFTN <b>C</b> GLL <b>C</b> YESKR-CONH <sub>2</sub>	1772.91	1772.91

<sup>a</sup> Cysteine residues and the corresponding serine residue in P1-Ser are highlighted in red.

### Arylation of Model Peptides

#### *Evaluation of Reaction Conditions for Peptide Arylation*



**Table S3. Reaction condition evaluation.<sup>a</sup>**

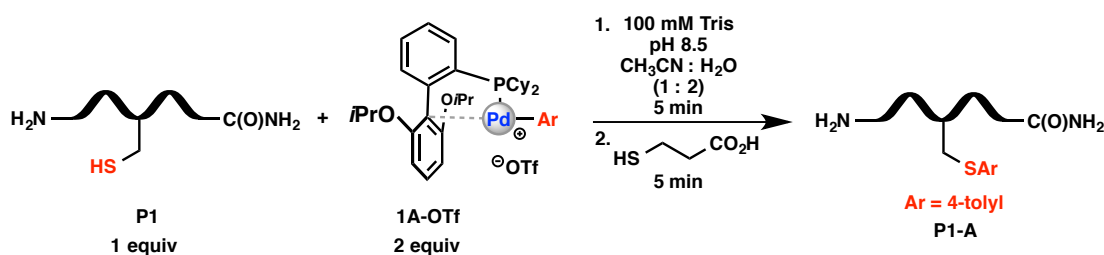
Entry	Buffer	Peptide Conc.	pH	Solvent	Product
1	100 mM Tris	1 mM	8.5	H <sub>2</sub> O:CH <sub>3</sub> CN (2:1)	93 %
2	100 mM Tris	<b>100 μM</b>	8.5	H <sub>2</sub> O:CH <sub>3</sub> CN ( <b>95:5</b> )	85 %
3	100 mM Tris	<b>10 μM</b>	<b>8.5</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
4	100 mM Tris	10 μM	<b>8</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
5	100 mM Tris	10 μM	<b>7.5</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
6	100 mM <b>HEPES</b>	10 μM	7.5	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
7	100 mM <b>MOPS</b>	10 μM	7.5	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
8	100 mM <b>Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub></b>	10 μM	7.5	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
9	<b>25 mM Tris</b>	10 μM	7.5	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	93 %

10	100 mM Tris	10 $\mu$ M	<b>7</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	84 %
11 <sup>b</sup>	<b>200 mM Tris</b>	10 $\mu$ M	<b>7</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	91 %
12	<b>100 mM MOPS</b>	10 $\mu$ M	<b>7</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
13	100 mM MOPS	10 $\mu$ M	<b>6.5</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
14	100 mM MES	10 $\mu$ M	<b>5.5</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	95 %
15 <sup>c</sup>	100 mM MES	10 $\mu$ M	<b>5.5</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
16	0.1 % TFA	10 $\mu$ M	<b>2.0</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	18 %
17 <sup>d</sup>	0.1 % TFA	10 $\mu$ M	<b>2.0</b>	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	59 %
18	100 mM Tris	10 $\mu$ M	7.5	H <sub>2</sub> O: <b>DMF</b> (95:5)	>99 %
19	100 mM Tris	10 $\mu$ M	7.5	H <sub>2</sub> O: <b>DMSO</b> (95:5)	>99 %
20 <sup>e</sup>	100 mM Tris	10 $\mu$ M	7.5	H <sub>2</sub> O:CH <sub>3</sub> CN (95:5)	>99 %
21 <sup>f</sup>	100 mM Tris	1 mM	8.5	H <sub>2</sub> O:CH <sub>3</sub> CN (2:1)	0 %

<sup>a</sup>Optimal conditions used for further substrate scope evaluation are highlighted in grey;

<sup>b</sup>Further increase in the molarity of the Tris buffer (400 mM) did not have an affect on the product yield. <sup>c</sup>Reaction time: 10 min; <sup>d</sup>Reaction time: 7 h 20 min; <sup>e</sup>Reaction performed in the presence of TCEP (20  $\mu$ M); <sup>f</sup>Peptide **P1-Ser** was used as the control.

### Procedures and LC-MS Traces for Experiments in Table S3



**General Procedure A (Table S3, Entries 3–7, 10, 13–15, 18, 19).** Peptide **P1** (4  $\mu$ L, 150  $\mu$ M in water), H<sub>2</sub>O (47  $\mu$ L), organic solvent (1  $\mu$ L), and the buffer (6  $\mu$ L, 1 M) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed by vortexing for 10 s. A stock solution of the palladium complex (2  $\mu$ L, 600  $\mu$ M) in organic solvent was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 5 min. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu$ L, 0.05  $\mu$ L/mL solution in water, 3 equiv to the palladium complex). After an additional 5 min, a

solution of 50% A : 50% B (v/v, 60  $\mu$ L) was added to the Eppendorf and the reaction mixture was analyzed by LC-MS.

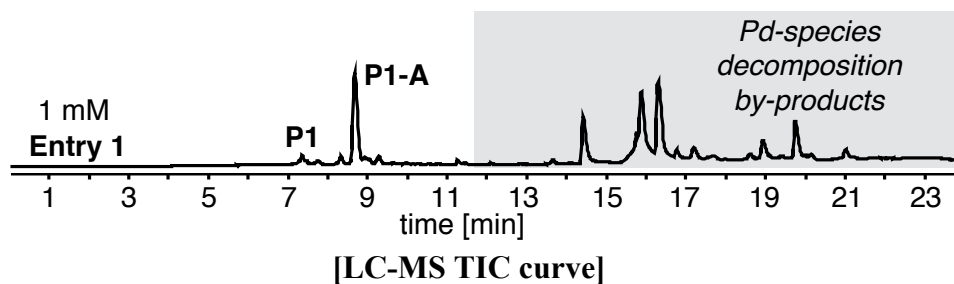
Final concentrations of the reaction before quenching:

peptide **P1** – 10  $\mu$ M, **Pd-complex** – 20  $\mu$ M, Buffer – 100 mM; organic solvent : H<sub>2</sub>O = 5 : 95.

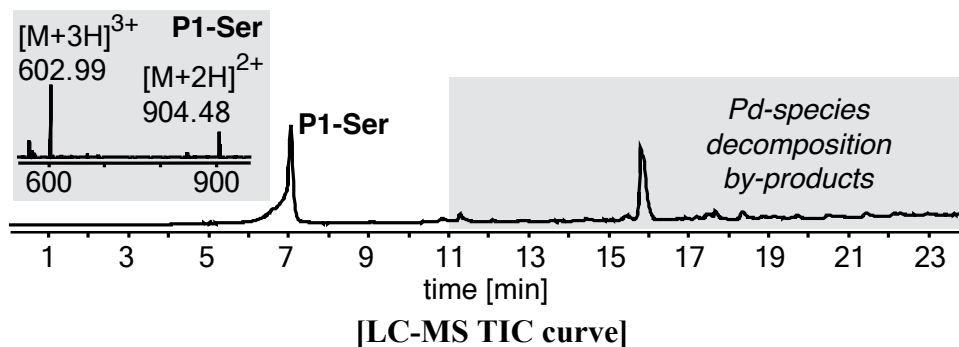
➤ *Effect of concentrations of peptides and palladium complexes (Entries 1–3).*

**Procedure for entries 1 and 21.** Peptide **P1** or **P1-Ser** (4  $\mu$ L, 15 mM in water), H<sub>2</sub>O (32  $\mu$ L), and Tris base buffer (4  $\mu$ L, 1 M, pH 8.5) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed by vortexing for 10 s. A stock solution of complex **1A-OTf** (20  $\mu$ L, 6 mM) in CH<sub>3</sub>CN was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 5 min. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu$ L, 5  $\mu$ L/mL solution in water, 3 equiv to **1A-OTf**). After an additional 5 min a solution of 50% A : 50% B (v/v, 550  $\mu$ L) was added to the Eppendorf and the reaction mixture was analyzed by LC-MS.

Final concentration of the reaction before quenching: peptide – 1 mM, **1A-OTf** – 2 mM, Tris buffer – 100 mM; CH<sub>3</sub>CN : H<sub>2</sub>O = 1 : 2.



➤ *Control (Entry 21):*

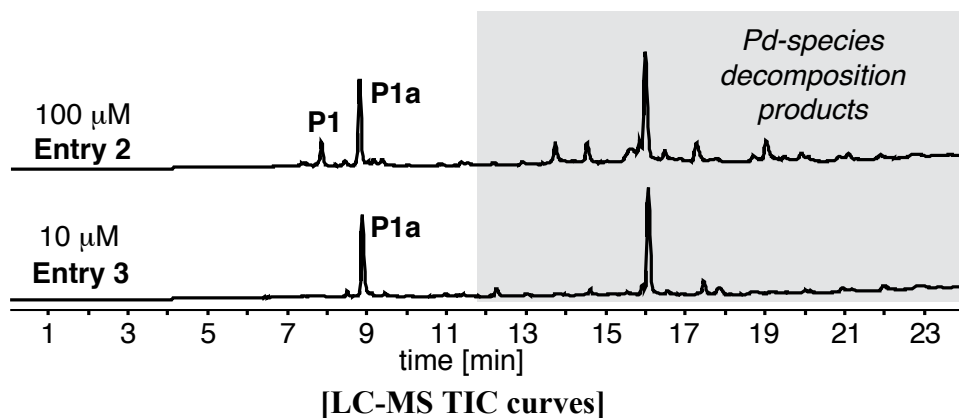


**Procedure for entry 2.** Peptide **P1** (4  $\mu$ L, 1.5 mM in water), H<sub>2</sub>O (47  $\mu$ L), CH<sub>3</sub>CN (1  $\mu$ L), and Tris buffer (6  $\mu$ L, 1 M, pH 8.5) were combined in a 0.6 mL plastic Eppendorf tube and the

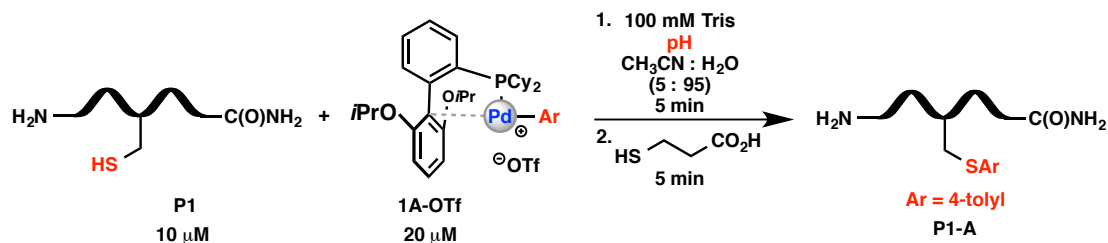
resulting solution was mixed by vortexing for 10 s. A stock solution of the palladium complex (2  $\mu\text{L}$ , 6 mM) in  $\text{CH}_3\text{CN}$  was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 5 min. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu\text{L}$ , 0.5  $\mu\text{L}/\text{mL}$  solution in water, 3 equiv to the palladium complex). After an additional 5 min, a solution of 50% A : 50% B (v/v, 60  $\mu\text{L}$ ) was added to the Eppendorf and the reaction mixture was analyzed by LC-MS.

Final concentrations of the reaction before quenching:

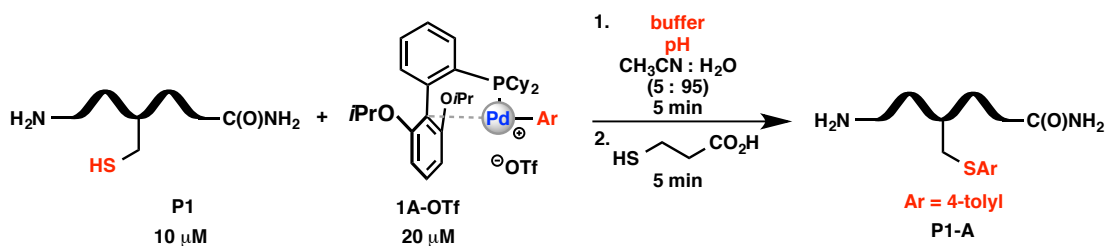
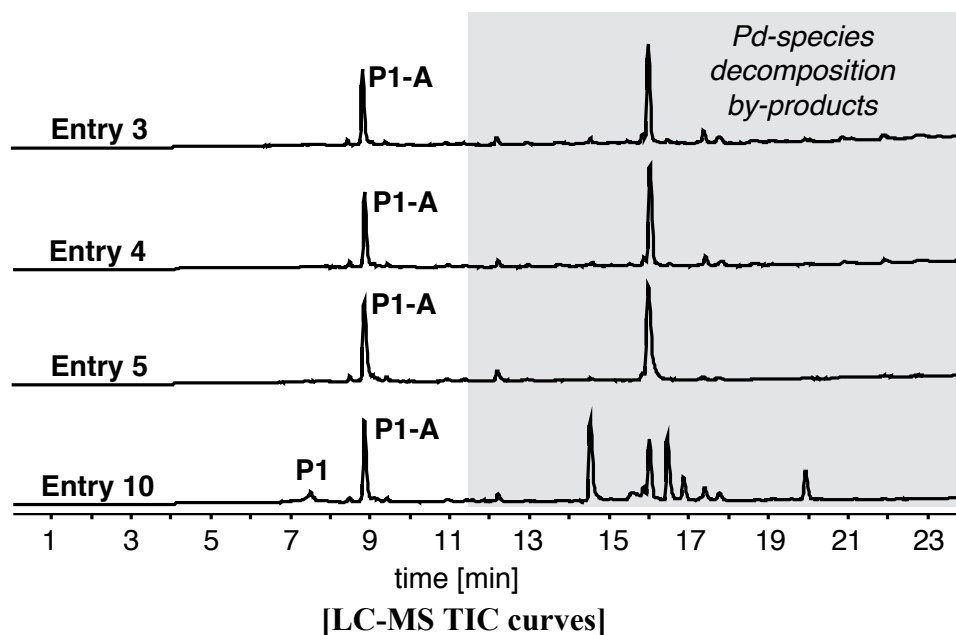
peptide – 100  $\mu\text{M}$ , **1A-OTf** – 200  $\mu\text{M}$ , Tris buffer – 100 mM;  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .



➤ Effect of pH on the bioconjugation reaction (Entries 3–5, 10–17).



✓ pH  $\geq$  7.0 (Entries 3–5, 10)



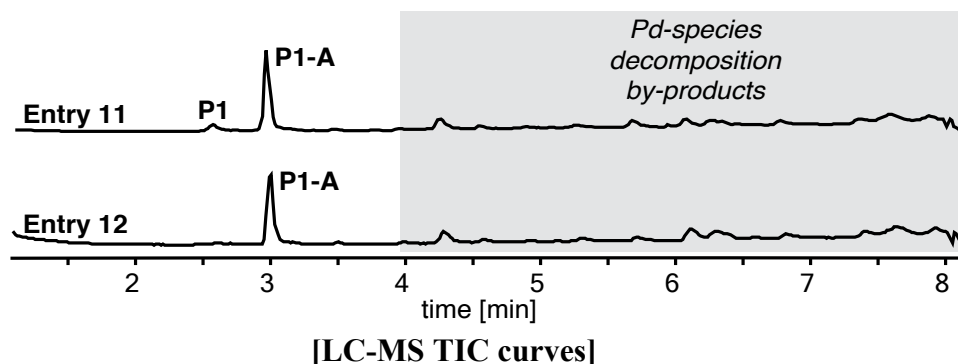
✓ pH 7.0 (Entries 11, 12)

**Experimental procedure for entries 11 and 12.** Peptide **P1** (6  $\mu$ L, 100  $\mu$ M in water), H<sub>2</sub>O (39  $\mu$ L), and the buffer (12  $\mu$ L, 1 M Tris or 0.5 M MOPS) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed by vortexing for 10 s. A stock solution of the palladium complex (3  $\mu$ L, 400  $\mu$ M) in CH<sub>3</sub>CN was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 5 min. The

reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu\text{L}$ , 0.05  $\mu\text{L}/\text{mL}$  solution in water, 3 equiv to the palladium complex). After an additional 5 min, a solution of 50% A : 50% B (v/v, 60  $\mu\text{L}$ ) was added to the Eppendorf and the reaction mixture was analyzed by LC-MS (*LC-MS data shown was acquired using Method C*)..

Final concentrations of the reaction before quenching:

peptide **P1** – 10  $\mu\text{M}$ , **Pd-complex** – 20  $\mu\text{M}$ , Buffer – 200 mM Tris or 100 mM MOPS; organic solvent :  $\text{H}_2\text{O}$  = 5 : 95.

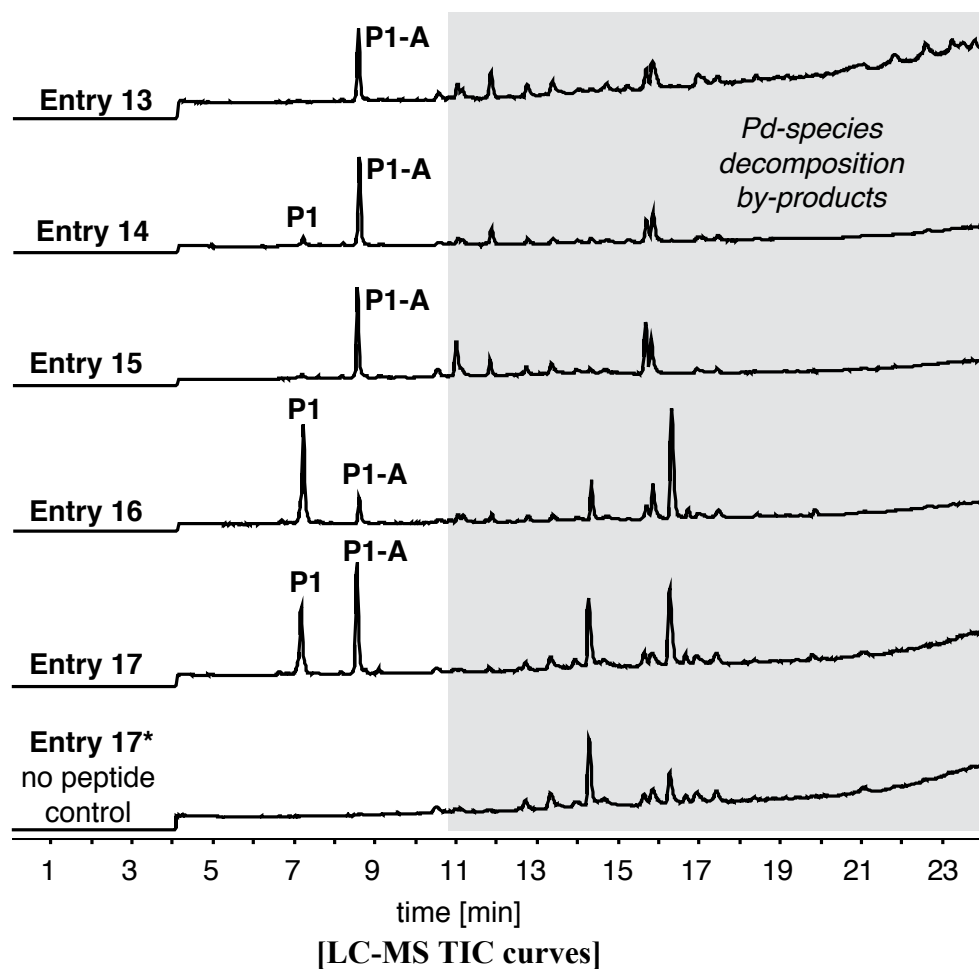


✓ pH < 7.0 (Entries 13–17)

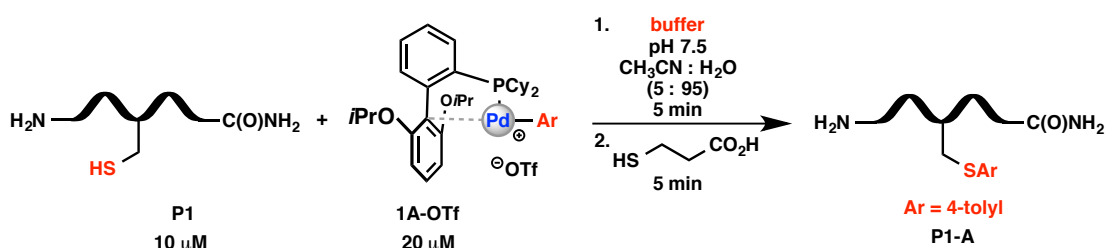
**Experimental procedure for entries 16 and 17.** Peptide **P1** (4  $\mu\text{L}$ , 150  $\mu\text{M}$  in water), 0.1% TFA solution in  $\text{H}_2\text{O}$  (53  $\mu\text{L}$ ), and  $\text{CH}_3\text{CN}$  (1  $\mu\text{L}$ ) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed by vortexing for 10 s. A stock solution of **1A-OTf** (2  $\mu\text{L}$ , 600  $\mu\text{M}$ ) in organic solvent was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 5 min or 7 h 20 min. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu\text{L}$ , 0.05  $\mu\text{L}/\text{mL}$  solution in water, 3 equiv to **1A-OTf**). After an additional 5 min, a solution of 50% A : 50% B (v/v, 60  $\mu\text{L}$ ) was added to the Eppendorf and the reaction mixture was analyzed by LC-MS.

Final concentrations of the reaction before quenching:

peptide – 10  $\mu\text{M}$ , **1A-OTf** – 20  $\mu\text{M}$ , pH = 2.0,  $\text{CH}_3\text{CN}$  :  $\text{H}_2\text{O}$  = 5 : 95.



➤ *Effect of the buffer on the bioconjugation reaction (Entries 5–9).*



**Experimental procedure for entry 8.** Peptide **P1** (4  $\mu\text{L}$ , 150  $\mu\text{M}$  in water),  $\text{H}_2\text{O}$  (23  $\mu\text{L}$ ),  $\text{CH}_3\text{CN}$  (1  $\mu\text{L}$ ), and  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$  buffer (30  $\mu\text{L}$ , 0.2 M, pH 7.5) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed by vortexing for 10 s. A stock solution of **1A-OTf** (2  $\mu\text{L}$ , 600  $\mu\text{M}$ ) in  $\text{CH}_3\text{CN}$  was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 5 min. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu\text{L}$ , 0.05  $\mu\text{L/mL}$  solution in water, 3 equiv to **1A-OTf**). After an additional 5 min, a solution of 50% A : 50% B (v/v, 60  $\mu\text{L}$ )

was added to the Eppendorf and the reaction mixture was analyzed by LC-MS.

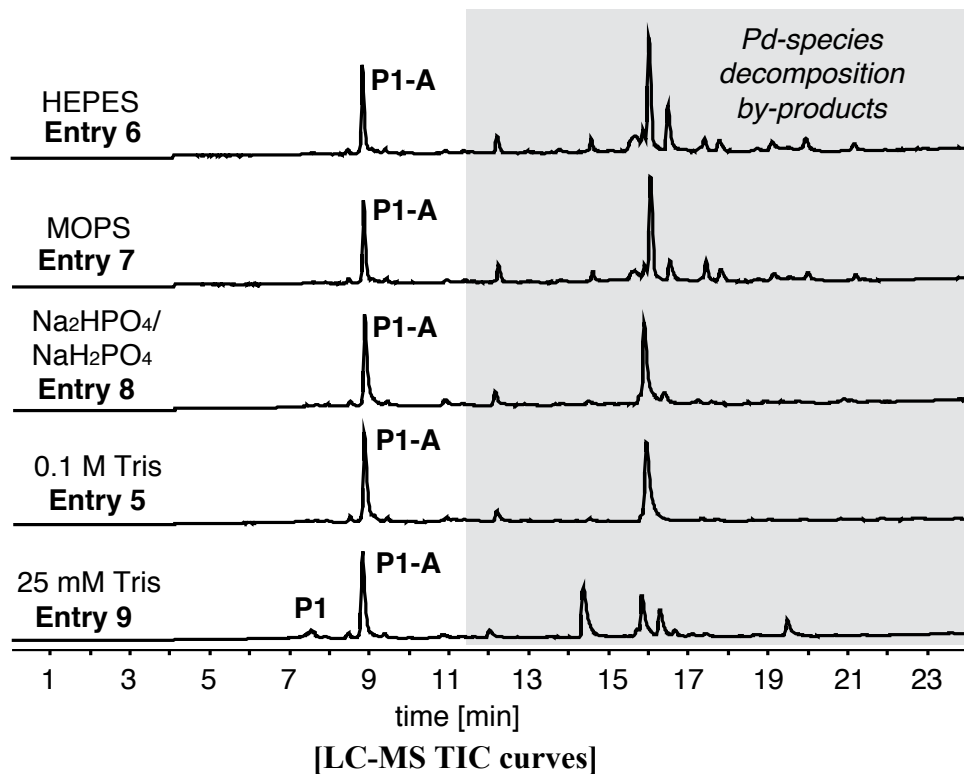
Final concentration of the reaction before quenching:

peptide – 10  $\mu$ M, **1A-OTf** – 20  $\mu$ M,  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$  – 100 mM;  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .

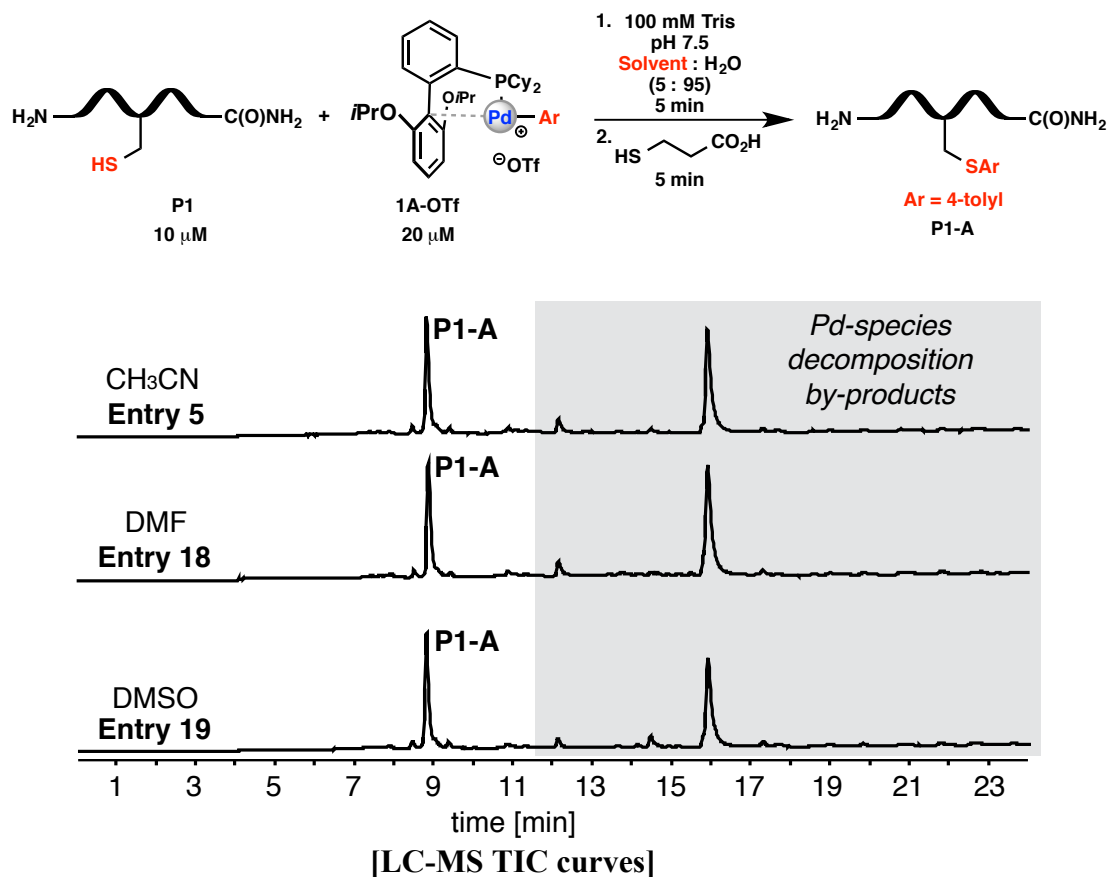
**Experimental procedure for entry 9.** Peptide **P1** (4  $\mu$ L, 150  $\mu$ M in water),  $\text{H}_2\text{O}$  (51.5  $\mu$ L),  $\text{CH}_3\text{CN}$  (1  $\mu$ L), and Tris buffer (1.5  $\mu$ L, 1 M, pH 7.5) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed by vortexing for 10 s. A stock solution of **1A-OTf** (2  $\mu$ L, 600  $\mu$ M) in  $\text{CH}_3\text{CN}$  was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 5 min. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu$ L, 0.05  $\mu$ L/mL solution in water, 3 equiv to **1A-OTf**). After an additional 5 min, a solution of 50% A : 50% B (v/v, 60  $\mu$ L) was added to the Eppendorf and the reaction mixture was analyzed by LC-MS.

Final concentration of the reaction before quenching:

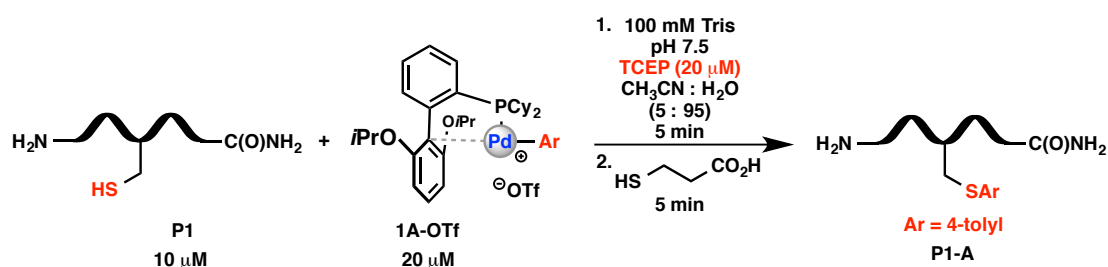
peptide – 10  $\mu$ M, **1A-OTf** – 20  $\mu$ M, Tris Buffer – 25 mM;  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .



➤ *Effect of organic solvent on the bioconjugation reaction (Entries 5, 18, 19).*



➤ *Reaction in the presence of TCEP (Entry 20).*

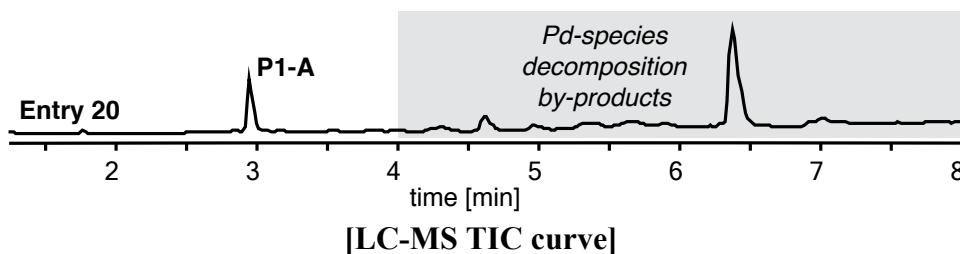


**Reaction procedure.** Peptide **P1** (4 μL, 150 μM in water), TCEP (2 μL, 600 μM), H<sub>2</sub>O (45 μL), CH<sub>3</sub>CN (1 μL), and Tris buffer (6 μL, 1 M, pH 7.5) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed by vortexing for 10 s. A stock solution of **1A-OTf** (2 μL, 600 μM) in CH<sub>3</sub>CN was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 5 min. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3 μL, 0.1 μL/mL solution in water, 6 equiv to **1A-OTf**). After an additional 5 min, a solution of 50% A : 50% B (v/v; 60 μL) was added to the

Eppendorf and the reaction mixture was analyzed by LC-MS (*LC-MS data shown was acquired using Method C*).

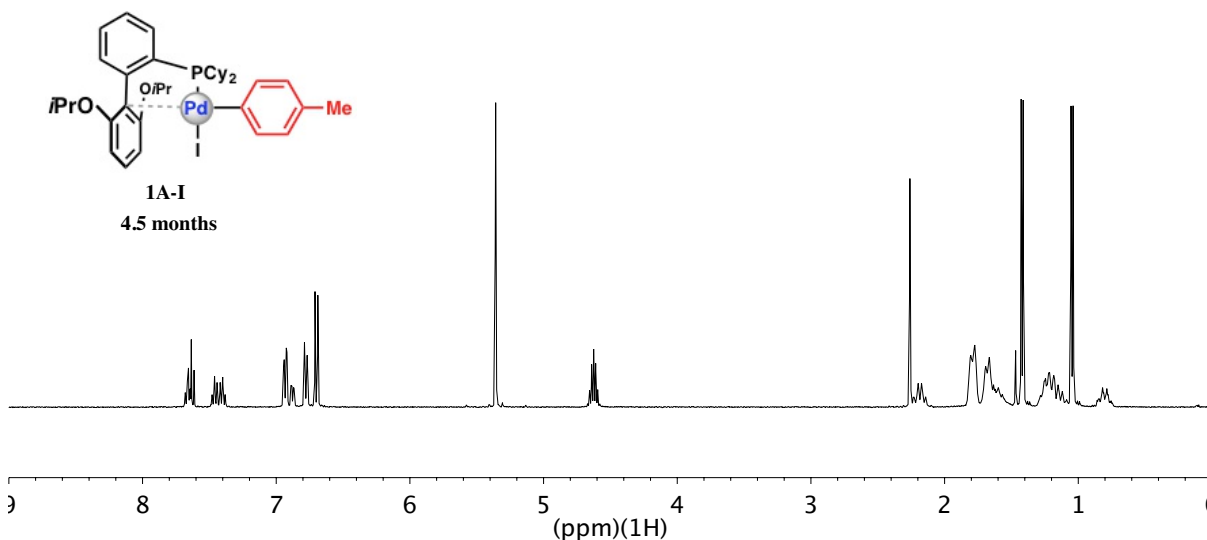
Final concentration of the reaction before quenching:

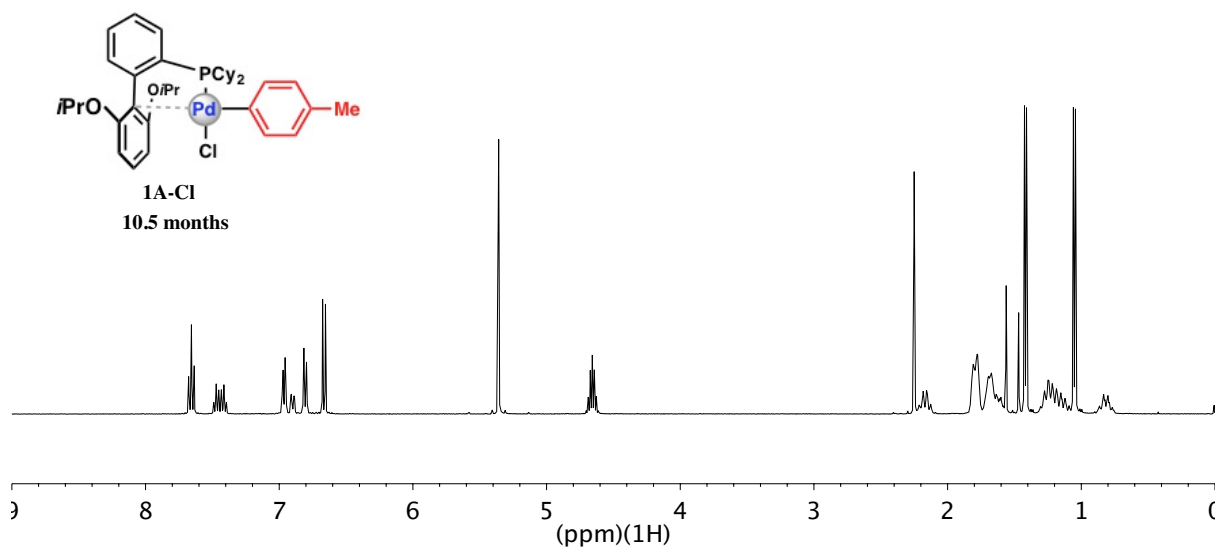
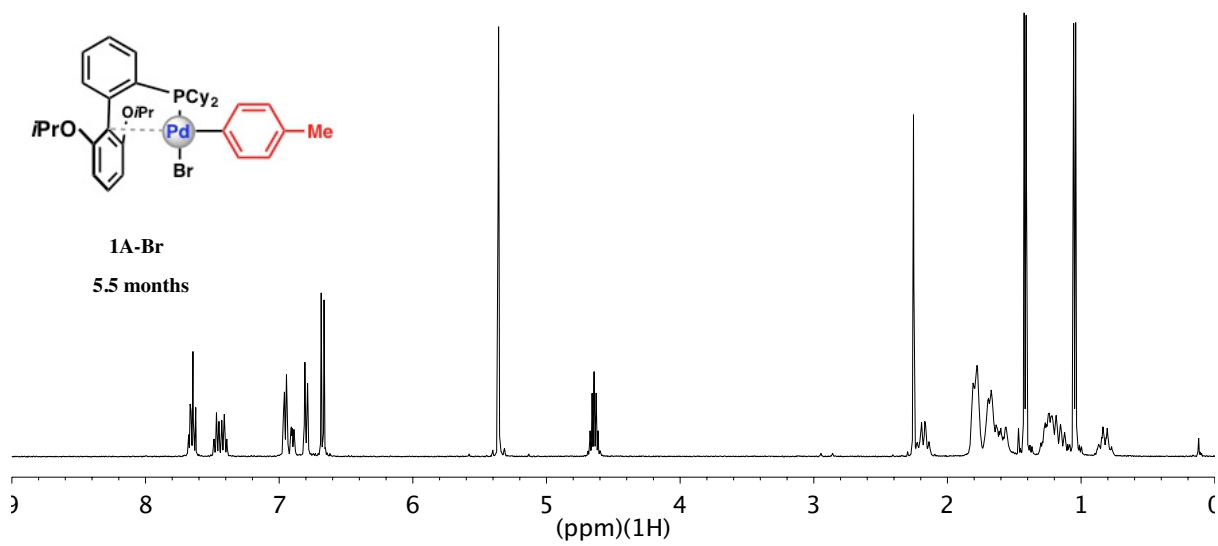
peptide – 10  $\mu$ M, **1A-OTf** – 20  $\mu$ M, TCEP – 20  $\mu$ M; Tris buffer – 100 mM; CH<sub>3</sub>CN : H<sub>2</sub>O = 5 : 95.

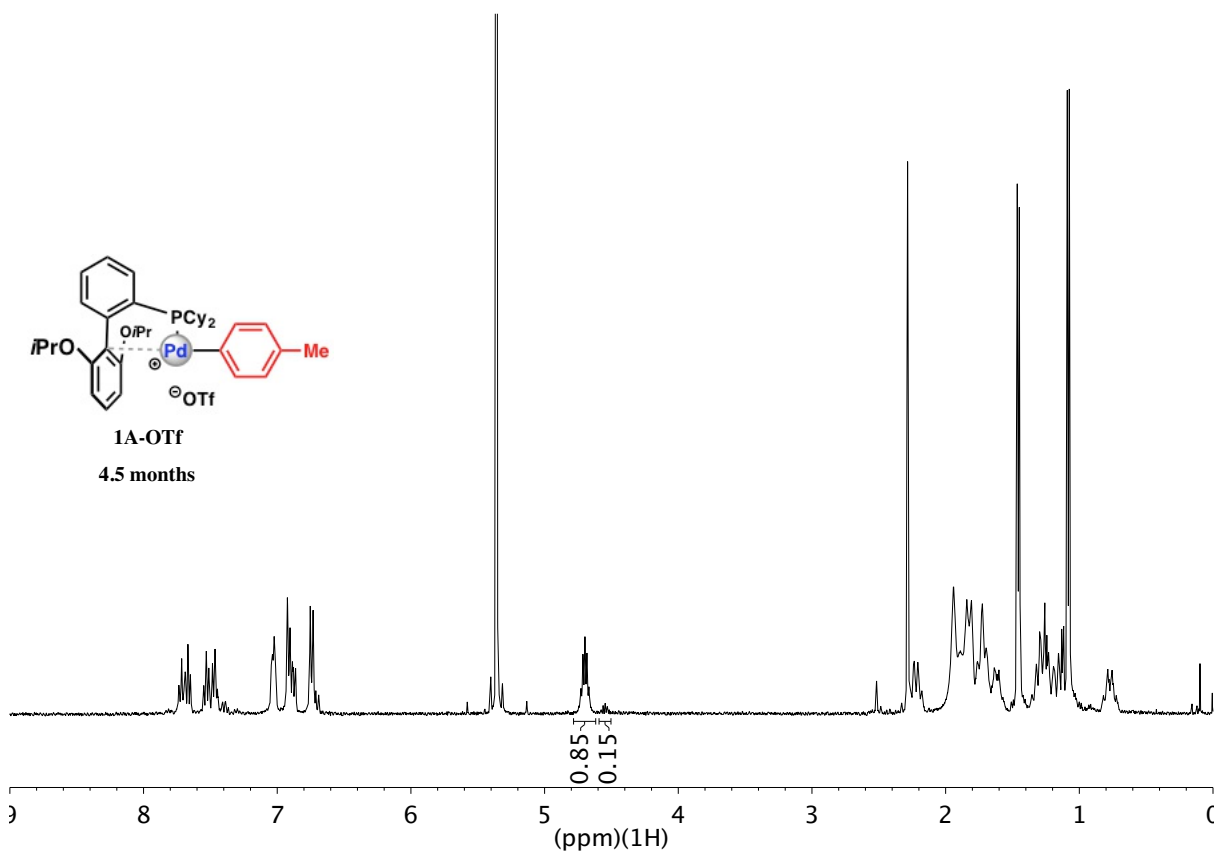


### Stability of the Oxidative Addition Complexes

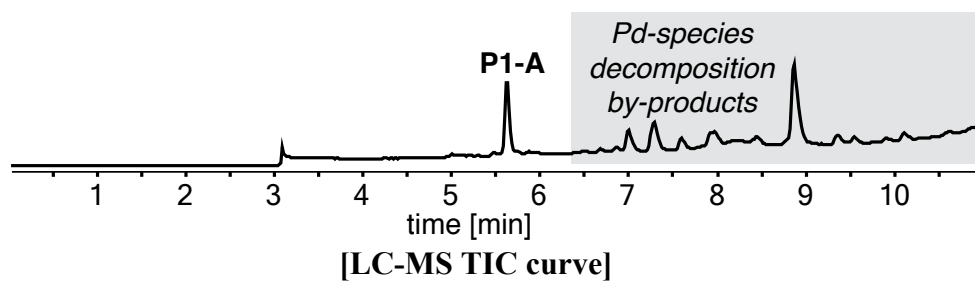
All synthesized palladium(II) complexes were stored in closed 5 mL scintillation vials under air at 4 °C. The long-term stability (4.5 – 10.5 months) of the 4-tolyl complexes (**1A-I**, **1A-Br**, **1A-Cl**, and **1A-OTf**) was evaluated by <sup>1</sup>H NMR spectroscopy in CD<sub>2</sub>Cl<sub>2</sub>. Only **1A-OTf** showed some decomposition after 4.5 months of storage in air (15% of a new RuPhos-containing by-product was detected). The reactivity of this "aged" complex was tested under standard conditions for cysteine arylation of peptide **P1** (Procedure A), and proved to be similar to the reactivity of the freshly made **1A-OTf** (Figure S1).



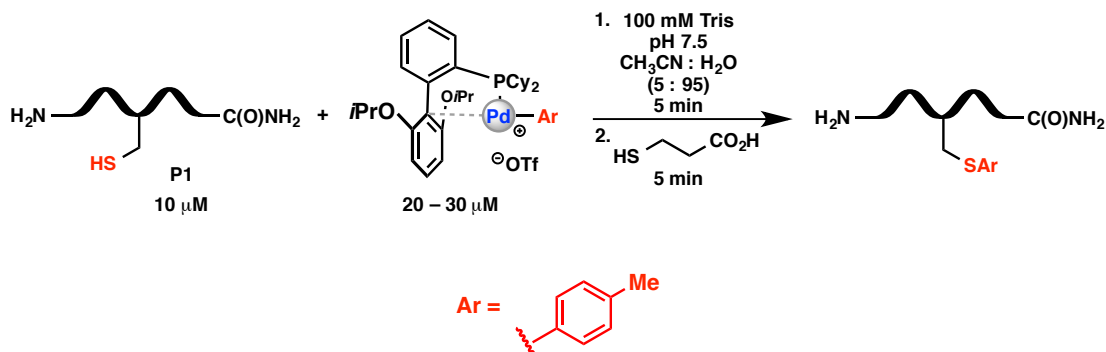




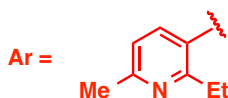
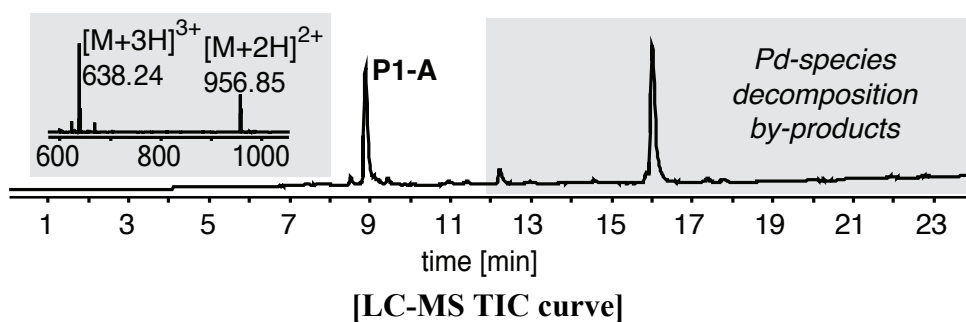
**Figure S1.** LC-MS TIC curve for the arylation of **P1** using "aged" **1A-OTf**. \* *LC-MS data shown was acquired using Method C.*



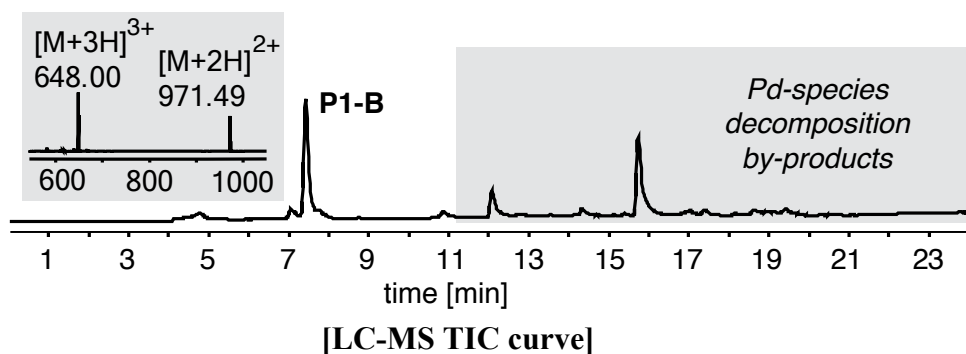
### Substrate Scope: Cysteine Bioconjugation with Different Palladium Reagents

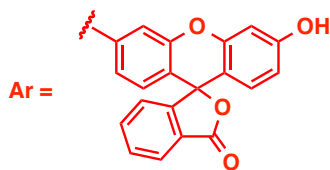


The modified peptide **P1-A** was synthesized according to standard procedure **A**. Final conditions before quenching: peptide – 10  $\mu\text{M}$ , **1A-OTf** – 20  $\mu\text{M}$ , 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .

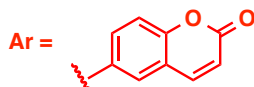
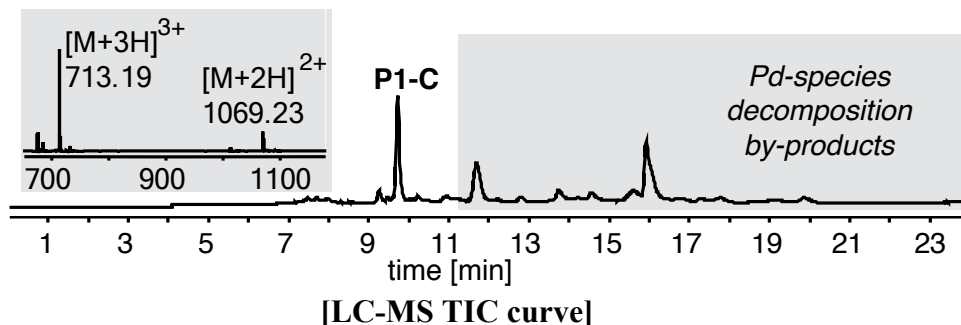


The modified peptide **P1-B** was synthesized according to standard procedure **A**. Final conditions before quenching: peptide – 10  $\mu\text{M}$ , **1B** – 20  $\mu\text{M}$ , 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .

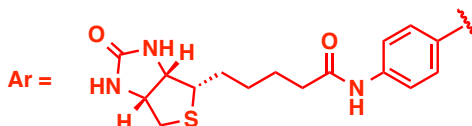
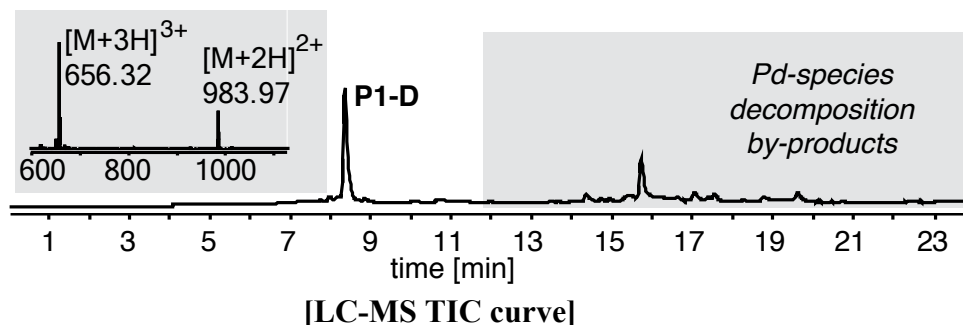




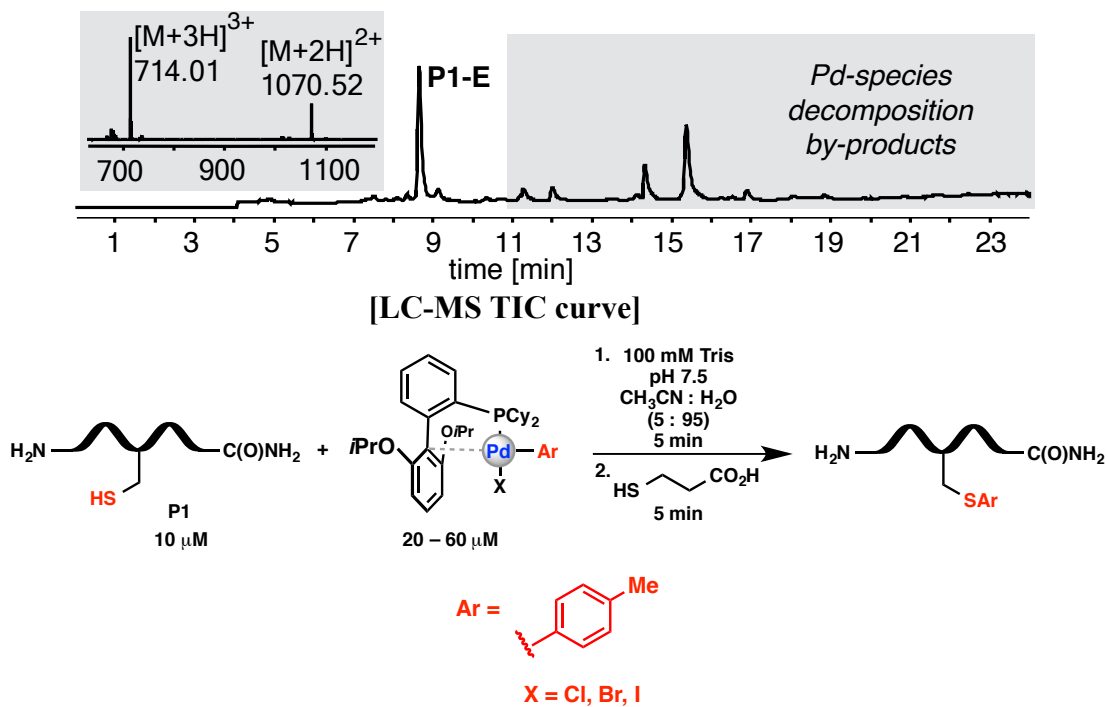
The modified peptide **P1-C** was synthesized according to standard procedure **A**. The reaction was quenched by the addition of 3-mercaptopropionic acid (12.5  $\mu\text{L}$ , 0.05  $\mu\text{L/mL}$  solution in water, 2 equiv to **1C**). Final conditions before quenching: peptide – 10  $\mu\text{M}$ , **1C** – 30  $\mu\text{M}$ , 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .



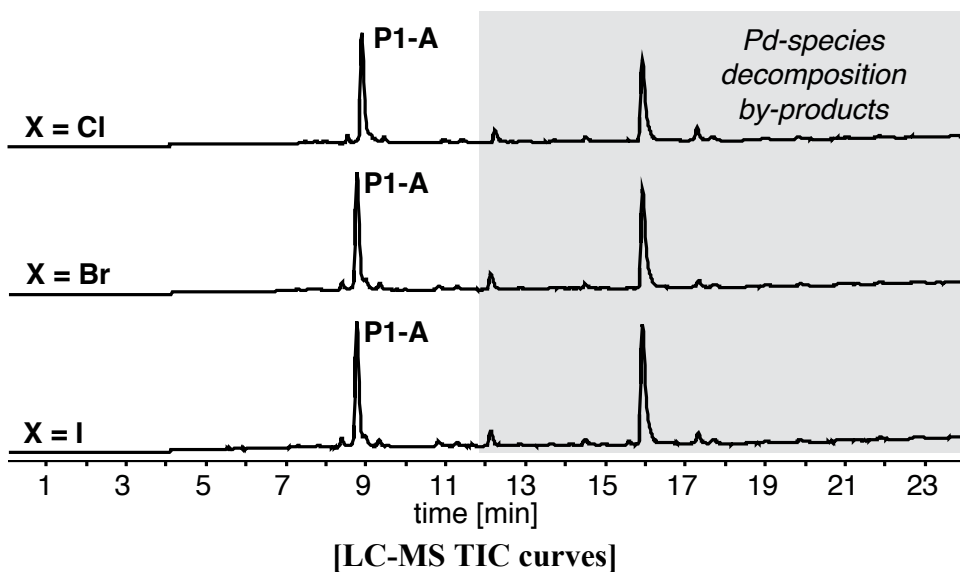
The modified peptide **P1-D** was synthesized according to standard procedure **A**. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu\text{L}$ , 0.05  $\mu\text{L/mL}$  solution in water, 2 equiv to **1D**). Final conditions before quenching: peptide – 10  $\mu\text{M}$ , **1D** – 30  $\mu\text{M}$ , 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .

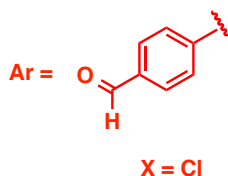


The modified peptide **P1-E** was synthesized according to standard procedure **A**. Final conditions before quenching: peptide – 10  $\mu\text{M}$ , **1E** – 20  $\mu\text{M}$ , 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .

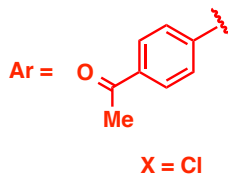
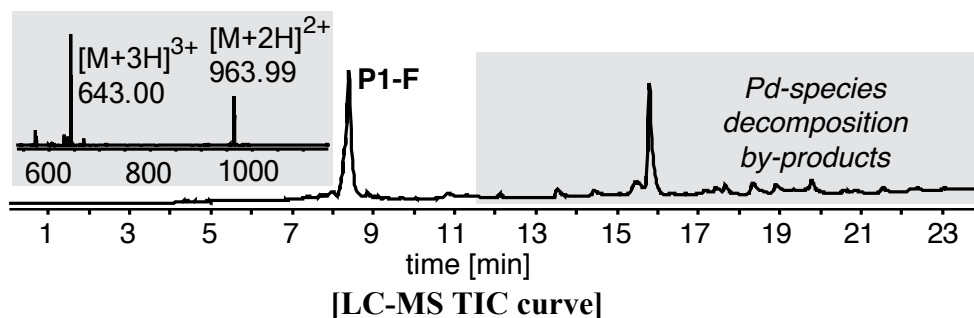


The modified peptide **P1-A** was synthesized according to standard procedure **A**. Final conditions before quenching: peptide – 10  $\mu$ M, **1A-X** (X = Cl, Br, I) – 20  $\mu$ M, 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .

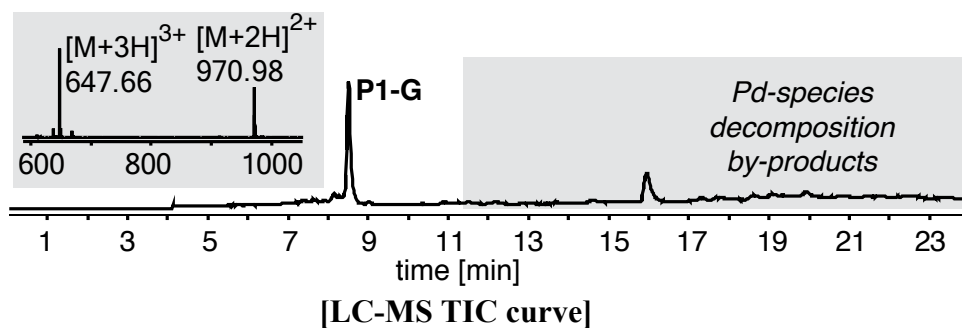


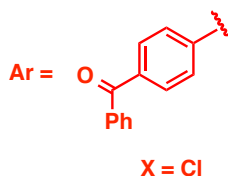


The modified peptide **P1-F** was synthesized according to standard procedure **A**. Final conditions before quenching: peptide – 10  $\mu\text{M}$ , **1F** – 20  $\mu\text{M}$ , 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ . **Note:** The product peak in the TIC contained a shoulder with the observed masses:  $[\text{M}+3\text{H}]^{3+} = 636.99$ ;  $[\text{M}+2\text{H}]^{2+} = 954.98$ . This data corresponds to a product with  $\text{M}_{\text{shoulder}} = [\text{M}_{\text{P1g}} - \text{H}_2\text{O}]$ , which can form as a result of reversible imine formation in the reaction between the aldehyde and the lysine residue within the peptide.

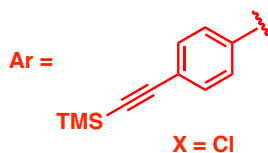
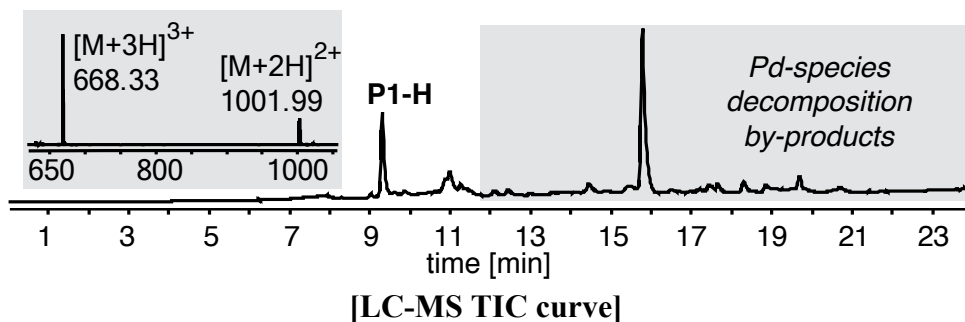


The modified peptide **P1-G** was synthesized according to standard procedure **A**. Final conditions before quenching: peptide – 10  $\mu\text{M}$ , **1G** – 20  $\mu\text{M}$ , 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .

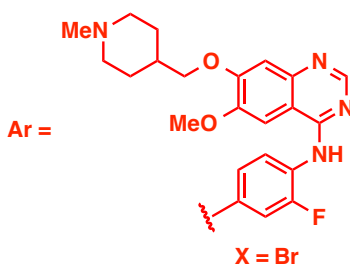
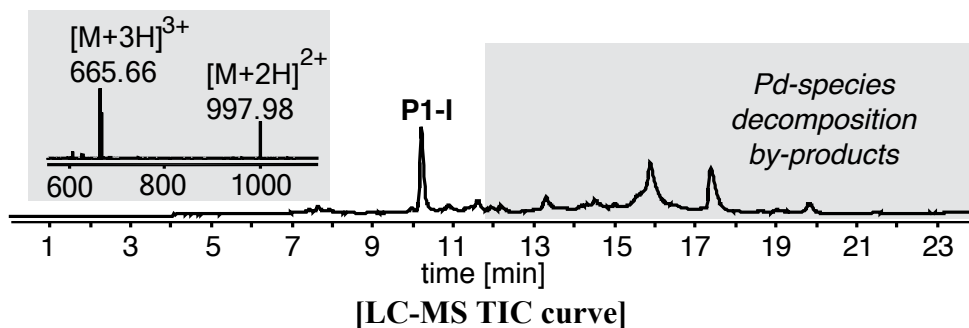




The modified peptide **P1-H** was synthesized according to standard procedure **A**. Final conditions before quenching: peptide – 10  $\mu\text{M}$ , **1H** – 20  $\mu\text{M}$ , 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .

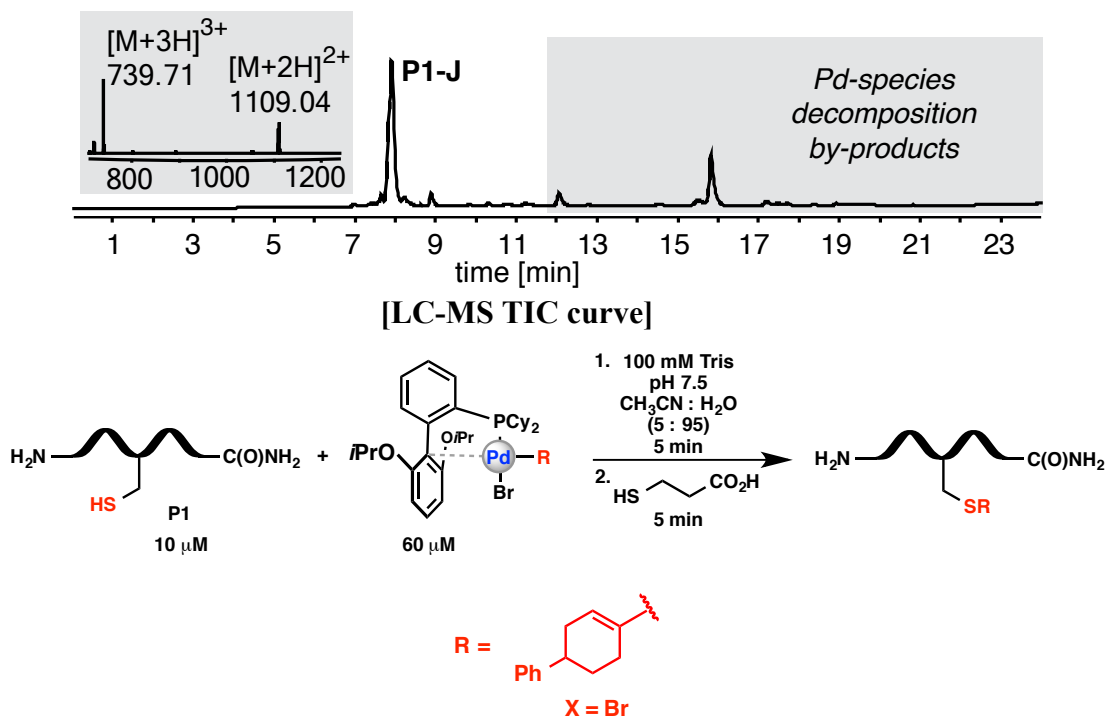


The modified peptide **P1-I** was synthesized according to standard procedure **A**. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu\text{L}$ , 0.05  $\mu\text{L}/\text{mL}$  solution in water, 1 equiv to **1I**). Final conditions before quenching: peptide – 10  $\mu\text{M}$ , **1I** – 60  $\mu\text{M}$ , 0.1 M Tris (pH 7.5),  $\text{CH}_3\text{CN} : \text{H}_2\text{O} = 5 : 95$ .

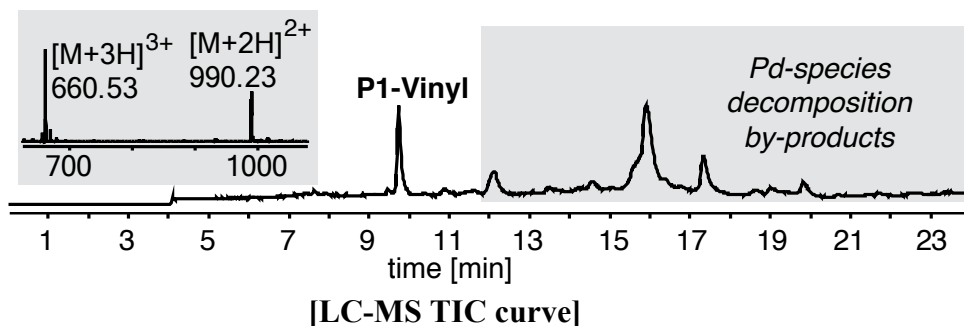


The modified peptide **P1-J** was synthesized according to standard procedure **A**. Final conditions

before quenching: peptide – 10  $\mu$ M, **1J** – 20  $\mu$ M, 0.1 M Tris (pH 7.5), CH<sub>3</sub>CN : H<sub>2</sub>O = 5 : 95.



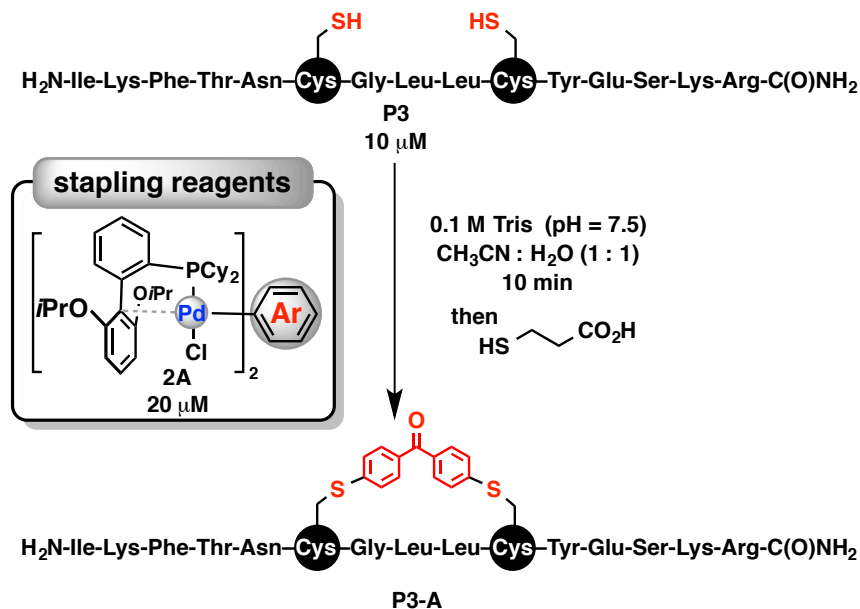
The modified peptide **P1-Vinyl** was synthesized according to standard procedure **A**. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu$ L, 0.05  $\mu$ L/mL solution in water, 1.5 equiv to **1-Vinyl**). Final conditions before quenching: peptide – 10  $\mu$ M, **1-Vinyl** – 40  $\mu$ M, 0.1 M Tris (pH 7.5), CH<sub>3</sub>CN : H<sub>2</sub>O = 5 : 95.



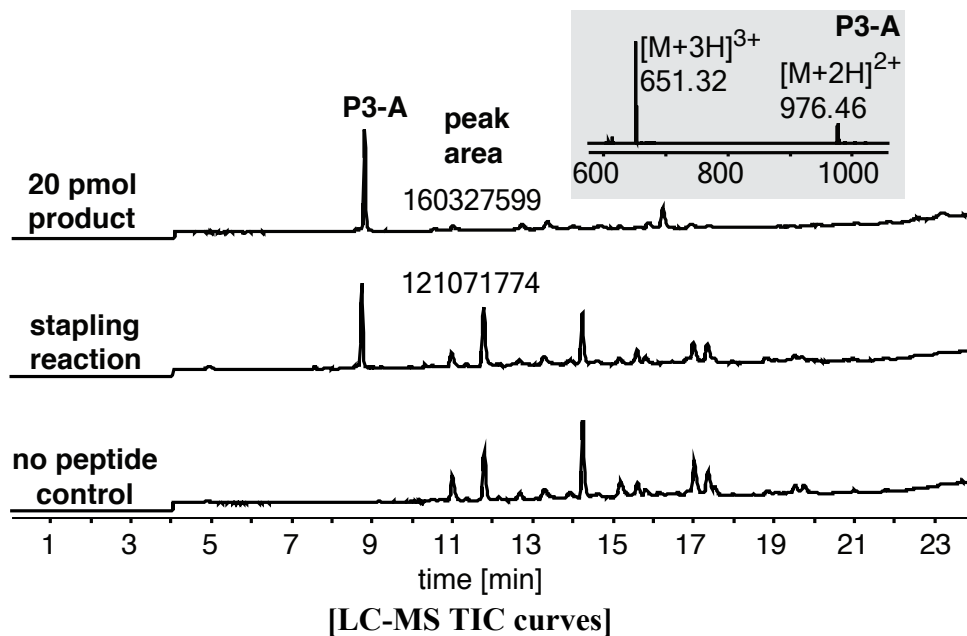
### Peptide Stapling.

Peptide **P3** (4  $\mu$ L, 150  $\mu$ M in water), H<sub>2</sub>O (23  $\mu$ L), and Tris buffer (3  $\mu$ L, 1 M, pH 7.5) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed by vortexing for 10 s. A stock solution of palladium complex **2A** (30  $\mu$ L, 40  $\mu$ M) in CH<sub>3</sub>CN was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 10 min. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu$ L, 0.1  $\mu$ L/mL solution in water, 6 equiv. to **2A**). After an additional 5 min a solution of

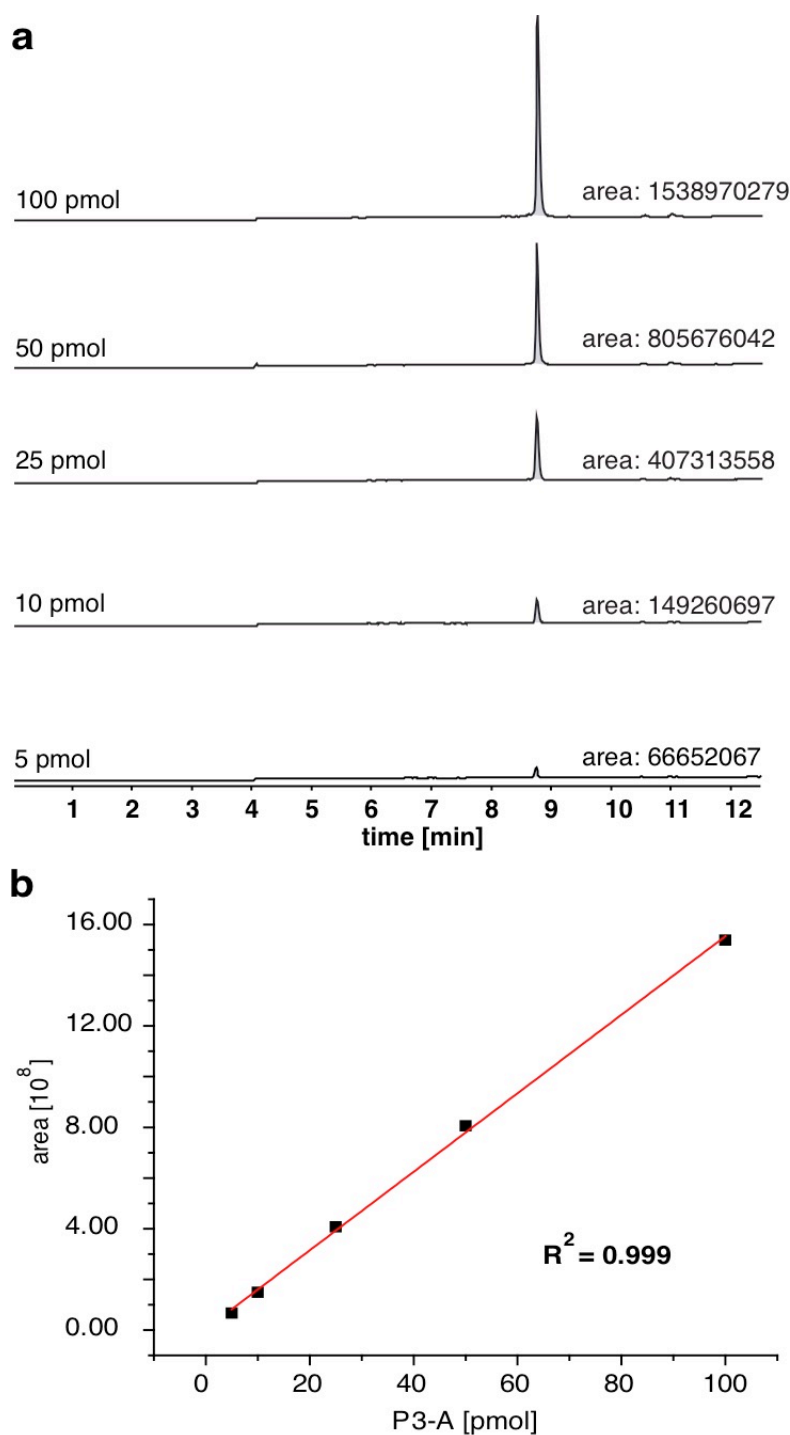
50% A : 50% B (v/v, 60  $\mu$ L) was added to the Eppendorf and the reaction mixture was analyzed by LC-MS (injection: 3  $\mu$ L).



Final concentration of the reaction before quenching: peptide **P3** – 10  $\mu$ M, **2A** – 20  $\mu$ M, Tris buffer – 100 mM; pH 7.5; CH<sub>3</sub>CN : H<sub>2</sub>O = 1 : 1.

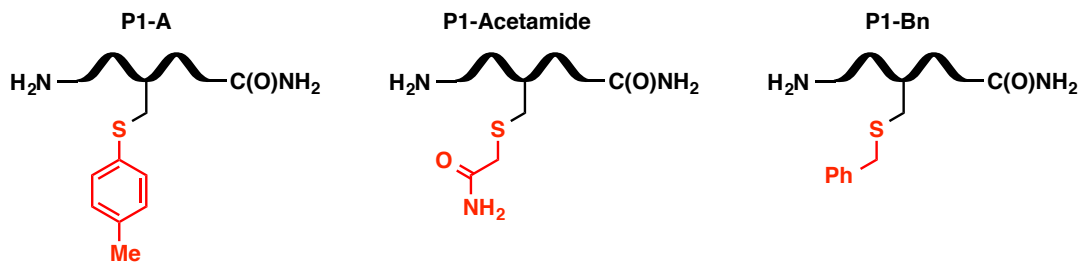


**Figure S2.** LC-MS standard curve for the stapled peptide product **P3-A**. (a) LC-MS chromatograms for analysis of various amount of **P3-A**. (b) Linear fitting of the amounts of **P3-A** to the area under the LC-MS TIC curves.

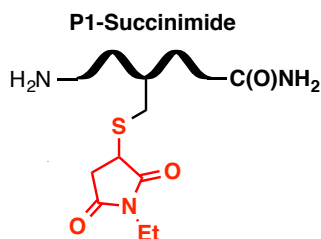


## Stability Evaluation of Peptide Cysteine Conjugates

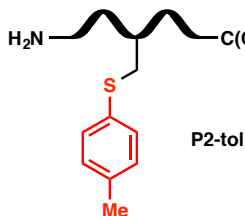
### Synthesis of Modified Peptides.



To a solution of peptide **P1** (3  $\mu$ moles) in Tris buffer (2 mL, 0.1 M, pH 8.0) in a 5-mL Eppendorf tube was added the corresponding labeling reagent (15  $\mu$ moles; **1A** for **P1-A**, iodoacetamide for **P1-Acetamide**, and benzylbromide for **P1-Bn**) dissolved in CH<sub>3</sub>CN (1 mL). The reaction was vortexed for 20 s to ensure proper reagent mixing and left at room temperature for 30 min. After this time, 20 mL of solvent A (0.1% TFA in water) was added to quench the reaction. The resulting solution was filtered and subjected to HPLC purification.

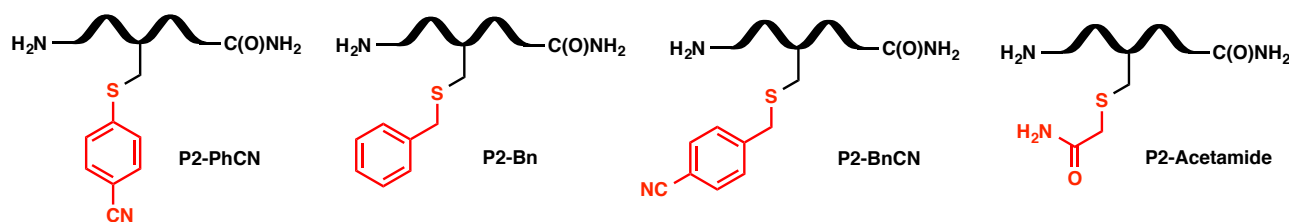


To a solution of peptide **P1** (3  $\mu$ moles) in MES buffer (2 mL, 0.1 M, pH 6.0) in a 5-mL Eppendorf tube was added *N*-ethyl maleimide (15  $\mu$ moles) dissolved in CH<sub>3</sub>CN (1mL). The reaction was vortexed for 20 s to ensure proper reagent mixing and left at room temperature for 30 min. After this time, 20 mL of solvent A (0.1% TFA in water) was added to quench the reaction. The resulting solution was filtered and subjected to HPLC purification.

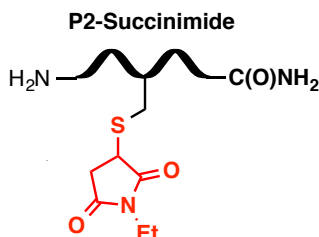


To a solution of peptide **P2** (9.5  $\mu$ moles) in Tris buffer (1.1 mL, 0.1 M, pH 8.0) in a 5-mL Eppendorf tube was added **1A** (19  $\mu$ moles) dissolved in CH<sub>3</sub>CN (1.1 mL). The reaction was

vortexed for 20 s to ensure proper reagent mixing and left at room temperature for 30 min. After this time, thiopropionic acid (30.6  $\mu\text{L}$  in 2 mL  $\text{H}_2\text{O}$ ) was added to quench the reaction. The resulting precipitate was spun down, separated from the solution and washed with an additional 1 mL of 0.1% TFA in water. The resulting solutions were combined and subjected to HPLC purification. **Note:** ICP-MS analysis of the pure isolated peptide showed 2.9 ppm remaining palladium content.



To a solution of peptide **P2** (10  $\mu\text{moles}$ ) in Tris buffer (1 mL, 0.1 M, pH 8.0) in a 5-mL Eppendorf tube was added the corresponding labeling reagent (11  $\mu\text{moles}$ ; **1-Benzonitrile** for **P2-PhCN**, benzylbromide for **P2-Bn**, *p*-cyanobenzylbromide for **P2-BnCN**, and iodoacetamide for **P2-Acetamide**) dissolved in  $\text{CH}_3\text{CN}$  (1 mL). The reaction was vortexed for 20 s to ensure proper reagent mixing and left at room temperature for 30 min. After this time, 10 mL of solvent A (0.1% TFA in water) was added to quench the reaction. The resulting solution was filtered and subjected to HPLC purification.



To a solution of peptide **P2** (10  $\mu\text{moles}$ ) in MES buffer (1 mL, 0.1 M, pH 6.0) in a 5-mL Eppendorf tube was added *N*-ethyl maleimide (11  $\mu\text{moles}$ ) dissolved in  $\text{CH}_3\text{CN}$  (1 mL). The reaction was vortexed for 20 s to ensure proper reagent mixing and left at room temperature for 30 min. 10 mL of solvent A (0.1% TFA in water) was added to quench the reaction. The resulting solution was filtered and then subjected to HPLC purification.

## 5. Stability Evaluation of Peptide Cysteine Conjugates

### Stability Evaluation in the Presence of Base, Acid or an External Thiol Nucleophile.

Peptide **P1** conjugates were pre-dissolved in water in plastic Eppendorfs to afford the 1.11 mM stock solutions used in the stability evaluation experiments. For each experiment, the corresponding cysteine conjugate (1.11 mM; 18  $\mu$ L) and stability test reagent (2  $\mu$ L, 50 mM in H<sub>2</sub>O or 50 mM in 1M Tris, pH 7.4) were combined in a plastic Eppendorf and left at rt for 2 days, followed by 4 days at 37 °C. After this time, individual reactions were quenched with a solution of 50% A : 50% B (v/v, 200 mL) and the resulting samples were analyzed by LC-MS (Figure S3 and Table S4).

#### **Basic conditions**

Stability test reagent: K<sub>2</sub>CO<sub>3</sub> (2  $\mu$ L, 50 mM in H<sub>2</sub>O);

Final conditions before quenching: 1mM peptide, 5 mM K<sub>2</sub>CO<sub>3</sub>; 2 d at rt, then 4 d at 37 °C.

#### **Acidic Conditions**

Stability test reagent: HCl (2  $\mu$ L, 1 M in H<sub>2</sub>O);

Final conditions before quenching: 1 mM peptide, 0.1 M HCl; 2 d at rt, then 4 d at 37 °C.

#### **Presence of External Thiol Nucleophiles: GSH**

Stability test reagent: Glutathione (2  $\mu$ L, 50 mM in 1 M Tris; pH 7.4);

Final conditions before quenching: 1 mM peptide, 5 mM GSH, 0.1M Tris, pH 7.4; 2 d at rt, then 4 d at 37 °C.

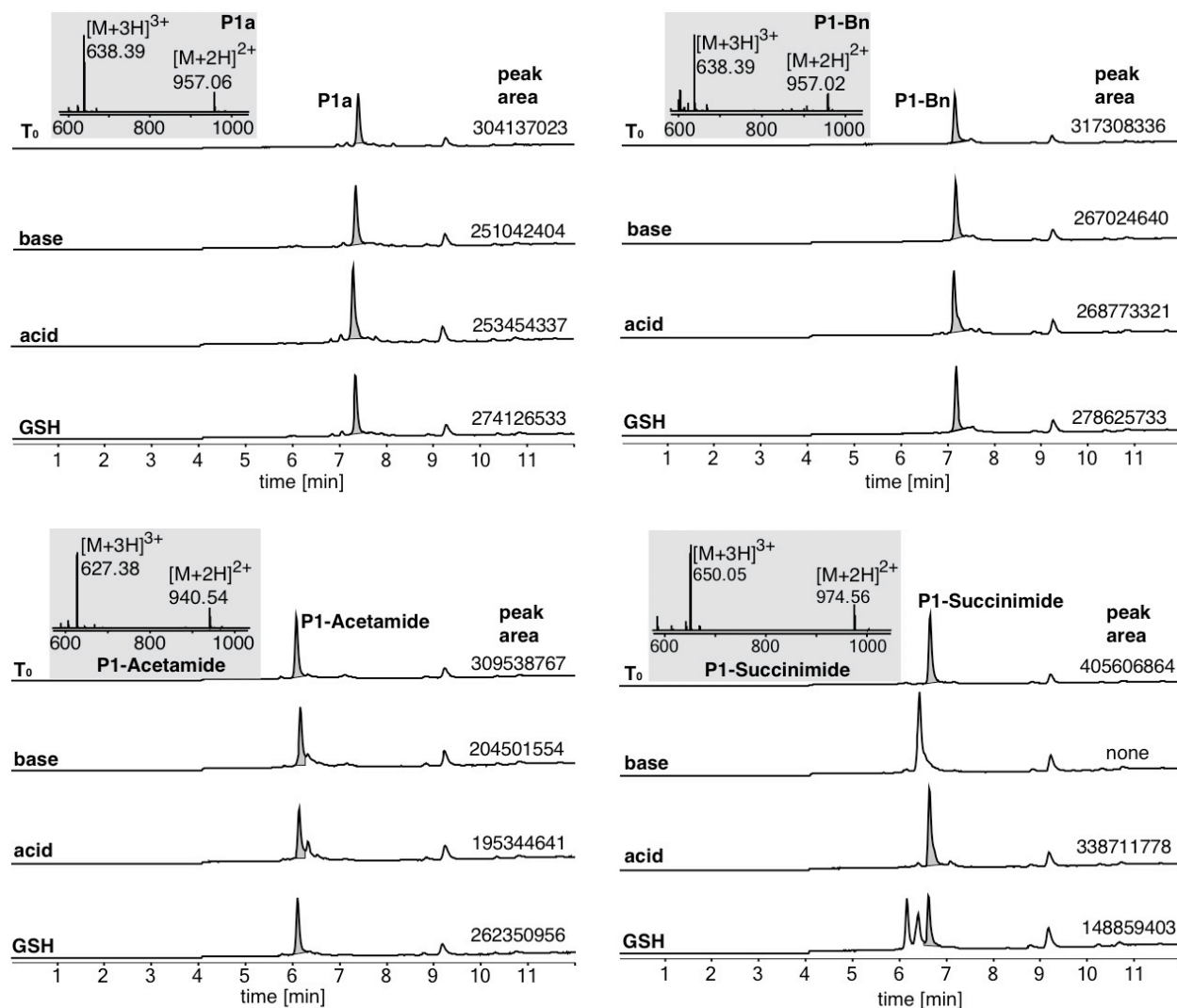
### Stability of Cysteine Conjugates toward Oxidation

Peptide **P2** conjugates were pre-dissolved in water in plastic Eppendorfs to afford the 111.1  $\mu$ M stock solutions used in the oxidation stability evaluation experiments. The corresponding cysteine conjugates (18  $\mu$ L, 111.1  $\mu$ M in H<sub>2</sub>O) and H<sub>5</sub>IO<sub>6</sub> (2  $\mu$ L, 4 mM in H<sub>2</sub>O) were then combined in a plastic Eppendorf, mixed using a vortexer and transferred into a pre-heated water bath at 37 °C. Individual reactions were quenched with Na<sub>2</sub>SO<sub>3</sub> (20  $\mu$ L, 4 mM in H<sub>2</sub>O) after 10 min, 30 min, 1 h, 2 h, 4 h, and 6 h, and the resulting mixtures were kept at rt for an

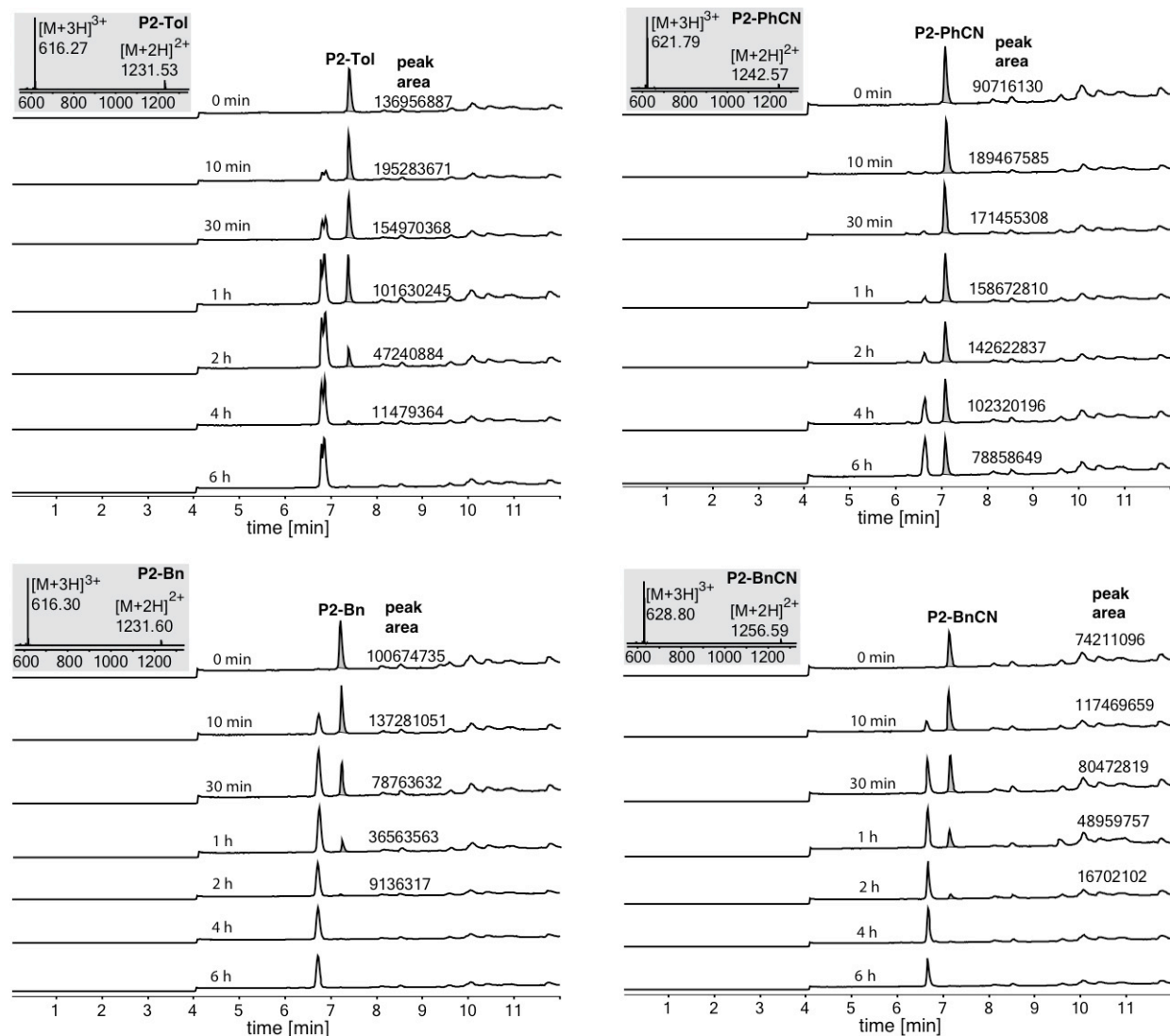
additional 10 min. Subsequently, a solution of 50% A : 50% B (v/v, 160  $\mu$ L) was added and the resulting samples were analyzed by LC-MS (Figure S4 and Table S5).

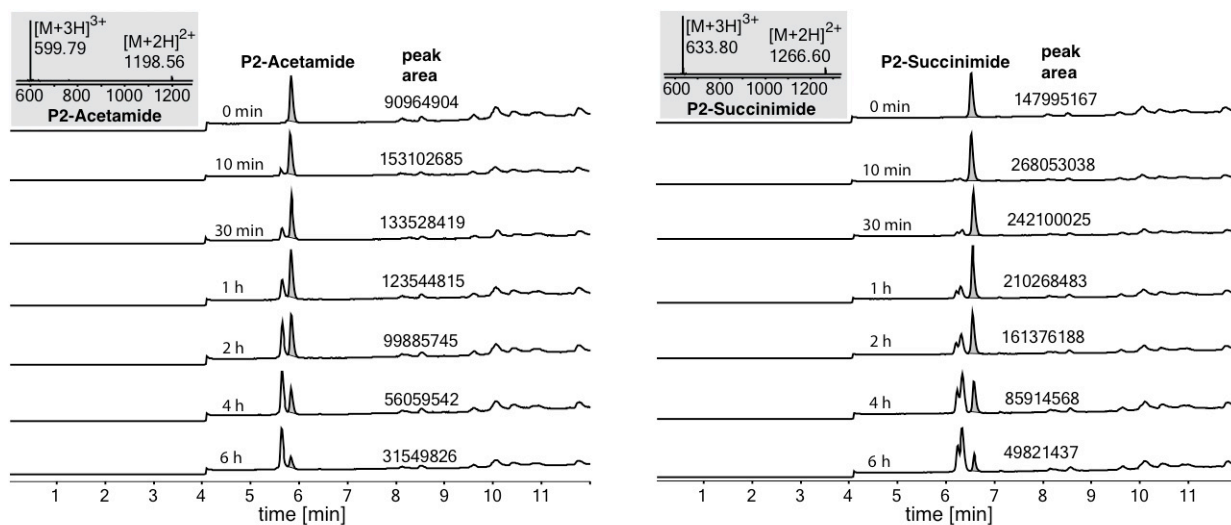
Final conditions before quenching: 100  $\mu$ M peptide, 400  $\mu$ M H<sub>3</sub>IO<sub>6</sub>, 37  $^{\circ}$ C.

**Figure S3.** LC-MS TIC curves for the experiments evaluating the stability of cysteine conjugates in the presence of base, acid, and external thiol nucleophiles (GSH). \* *LC-MS data shown were acquired using Method B.*



**Figure S4.** LC-MS TIC curves for the experiments evaluating the stability of cysteine conjugates under oxidative conditions. \* *LC-MS data shown were acquired using Method B.*



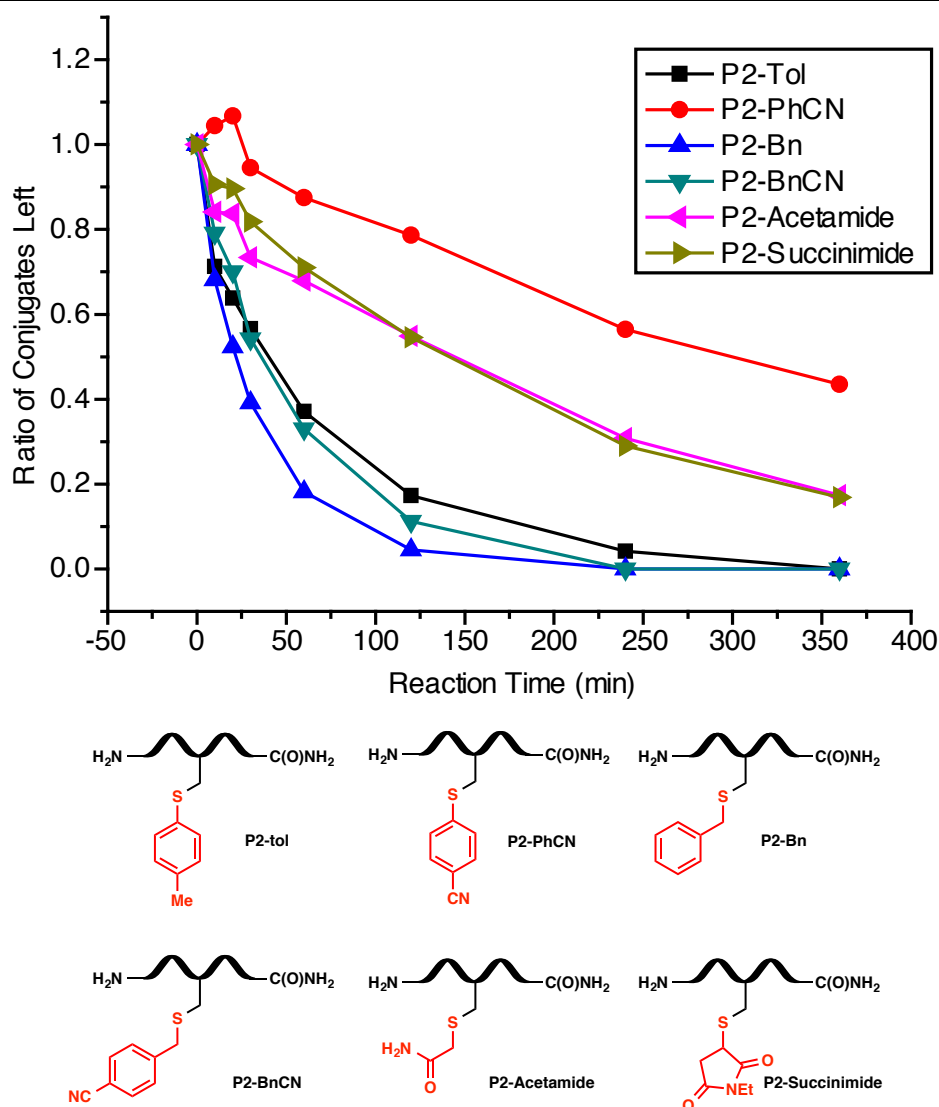


**Table S4.** Stability of the cysteine conjugates in the presence of base, acid, external thiol nucleophiles, and under oxidative conditions.

	A	B	C	D
	% remaining peptide			
base	83 %	0 %	66 %	84 %
acid	83 %	84 %	63 %	85 %
GSH	90 %	37 %	85 %	88 %
oxidation <sup>a</sup>	73 %	82 %	73 %	39 %

<sup>a</sup>Reaction time – 30 min.

**Table S5.** Stability of cysteine conjugates under oxidative conditions.



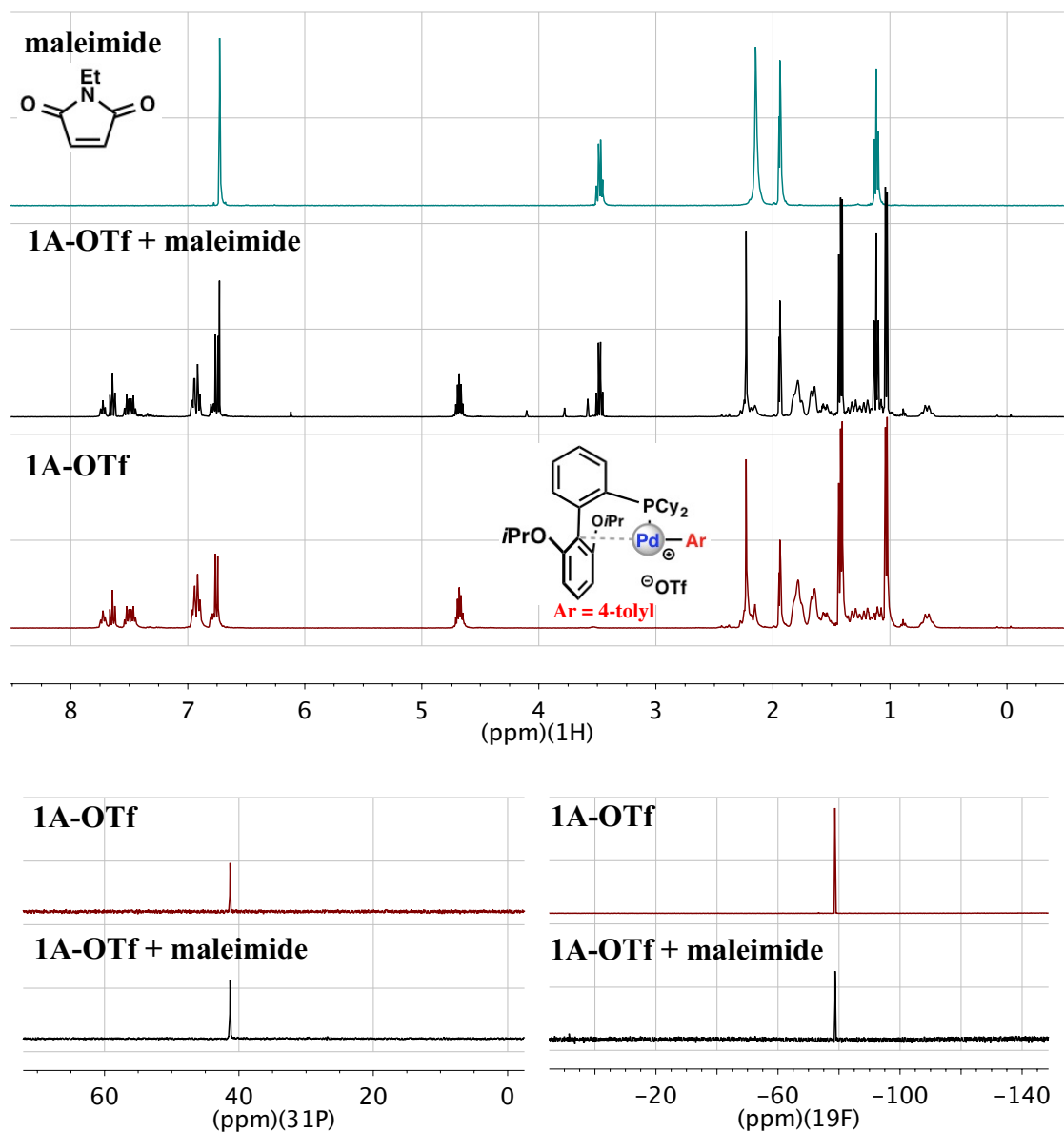
## 6. Evaluation of Reaction Kinetics

Kinetic parameters of the maleimide conjugation at biologically relevant pH are typically determined by extrapolation of the results obtained at lower pH, at which the reaction is significantly slower.<sup>12</sup> This approach could not be applied to our system due to potential variation of the reactive species structure at different pH. Both of the bioconjugation reactions are extremely fast and reach completion at room temperature at pH 7.5 in less than 30 s. Therefore, the rate of the process was estimated through a competition experiment between palladium reagent **1A-OTf** and *N*-ethyl maleimide at pH 7.5 and pH 5.5 in the reaction with

peptide **P2**. First, the orthogonality of the palladium complex and the maleimide reagent was established by dissolving equimolar quantities of *N*-ethyl maleimide and complex **1A-OTf** in CD<sub>3</sub>CN and subsequent analysis of the resulting solution by <sup>1</sup>H, <sup>31</sup>P and <sup>19</sup>F NMR spectroscopies (Fig. S5).

The relative reactivities of both bioconjugation reagents were further evaluated in competition experiments with peptide **P2** (Fig. S6).

**Figure S5.** Comparison of the <sup>1</sup>H, <sup>31</sup>P and <sup>19</sup>F NMR spectra of *N*-ethyl maleimide, palladium reagent **1A-OTf** and their equimolar mixture.



### Standard Bioconjugation Procedure for Competition Experiments.

Peptide **P2** (4  $\mu$ L, 150  $\mu$ M), H<sub>2</sub>O (47  $\mu$ L), Tris buffer (6  $\mu$ L, 1 M, pH 7.5 or pH 5.5), and CH<sub>3</sub>CN (1  $\mu$ L) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed using a vortexer. A stock solution of the bioconjugation reagent(s) (2  $\mu$ L, 600  $\mu$ M **N-ethyl maleimide**, 600  $\mu$ M **1A-OTf**, or [600  $\mu$ M **N-ethyl maleimide** and 600  $\mu$ M **1A-OTf**]) in CH<sub>3</sub>CN was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at room temperature for 5 min. The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3  $\mu$ L, 0.05  $\mu$ L/mL solution, 3 equiv to **1A-OTf**). After an additional 5 min a solution of 50% A : 50% B (v/v, 60 mL) was added to the Eppendorf and the reaction mixture was analyzed by LC-MS (Fig. S6).

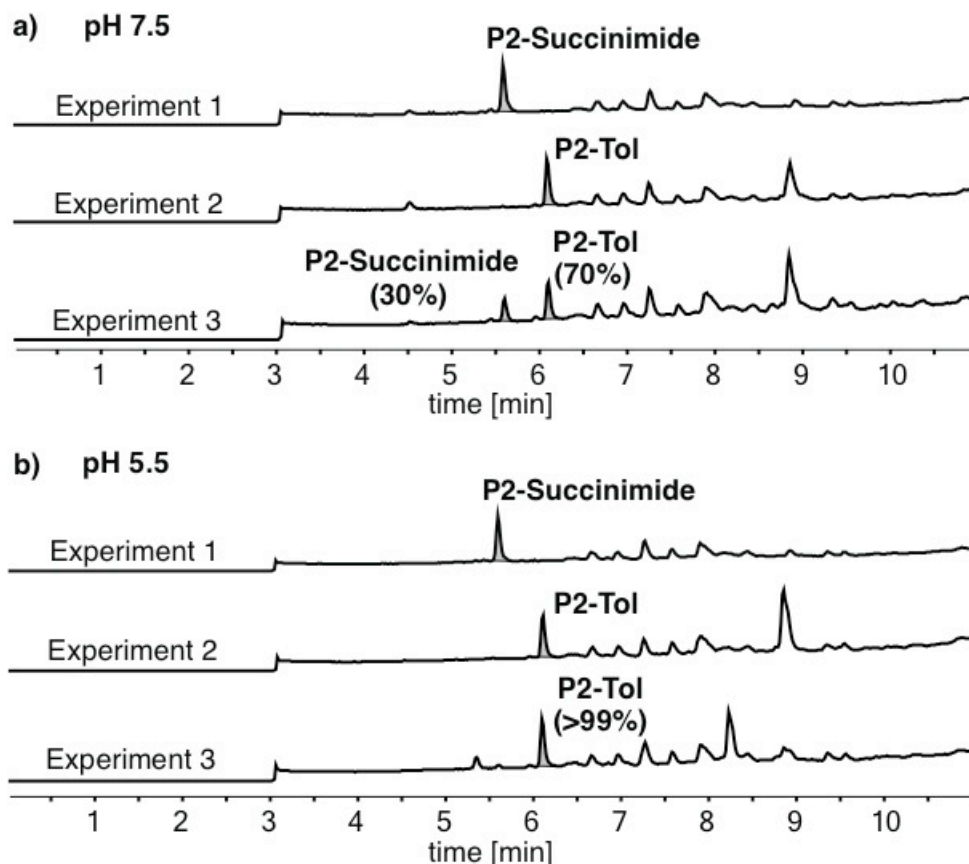
Final conditions before quenching:

Experiment 1. **P2** – 10  $\mu$ M, **N-ethyl maleimide** – 20  $\mu$ M, 0.1 M Tris (pH 7.5 or pH 5.5), CH<sub>3</sub>CN : H<sub>2</sub>O = 5 : 95.

Experiment 2. **P2** – 10  $\mu$ M, **1A-OTf** – 20  $\mu$ M, 0.1 M Tris (pH 7.5 or pH 5.5), CH<sub>3</sub>CN : H<sub>2</sub>O = 5 : 95.

Experiment 3. **P2** – 10  $\mu$ M, **1A-OTf** – 20  $\mu$ M, **N-ethyl maleimide** – 20  $\mu$ M, 0.1 M Tris (pH 7.5 or pH 5.5), CH<sub>3</sub>CN : H<sub>2</sub>O = 5 : 95. *Note: N-ethyl maleimide and 1A-OTf were added together as a solution in CH<sub>3</sub>CN.*

**Figure S6.** LC-MS TIC curves for the experiments evaluating relative rates of cysteine bioconjugation with *N*-ethyl maleimide and palladium reagent **1A-OTf** at (a) pH 7.5 and (b) pH 5.5. \*LC-MS data shown were acquired using Method B.



## 7. Protein Experiments

### *Protein Expression and Purification*

pET-SUMO-DARPin, pET-SUMO-10FN3, pET-SUMO-Affibody plasmids were constructed as reported previously.<sup>13</sup> Cysteine mutations were introduced by site-directed mutagenesis using QuickChange Lightning Single Site-directed Mutagenesis Kit (Agilent) following manufacturer's instructions. Sequences of generated protein constructs are summarized in Table S6.

*E. coli* BL21(DE3) cells transformed with pET-SUMO-Protein plasmid were grown in 1 L of LB medium containing kanamycin (30 µg/mL) at 37 °C until OD<sub>600</sub> = 0.6. Then, expression was induced by the addition of 0.5 mM IPTG overnight at 30 °C. After harvesting the cells by centrifugation (6,000 rpm for 10 min), the cell pellet was lysed by sonication in 25 mL of 50 mM

Tris and 150 mM NaCl (pH 7.5) buffer containing 15 mg lysozyme (Calbiochem), 1 mg DNase I (Sigma-Aldrich), and 0.5 tablet of protease inhibitor cocktail (Roche Diagnostics, Germany). The resulting suspension was centrifuged at 17,000 rpm for 30 min to remove cell debris. The supernatant was loaded onto a 5 mL HisTrap FF crude Ni-NTA column (GE Healthcare, UK), first washed with 40 mL of 20 mM Tris and 150 mM NaCl (pH 8.5), and then washed with 40 mL of 40 mM imidazole in 20 mM Tris and 150 mM NaCl (pH 8.5). The protein was eluted from the column with buffer containing 500 mM imidazole in 20 mM Tris and 150 mM NaCl (pH 8.5). Imidazole was removed from protein using a HiPrep 26/10 Desalting column (GE Healthcare, UK), the protein was eluted into 20 mM Tris and 150 mM NaCl (pH 7.5) buffer. The protein was analyzed by LC-MS to confirm its purity and molecular weight.

SUMO group on SUMO-Protein was cleaved by incubating 1  $\mu$ g of SUMO protease per mg of protein at room temperature for 60 min. The crude reaction mixture was loaded onto a 5 mL HisTrap FF crude Ni-NTA column (GE Healthcare, UK) and the flow through containing the desired protein was collected. The protein was analyzed by LC-MS confirming sample purity and molecular weight. Purified proteins were concentrated using Amicon 3K concentrator (50 mL, EMD Millipore); protein aliquots were flash frozen and stored in  $-80^{\circ}\text{C}$  freezer.

### ***Protein Labeling Experiments***

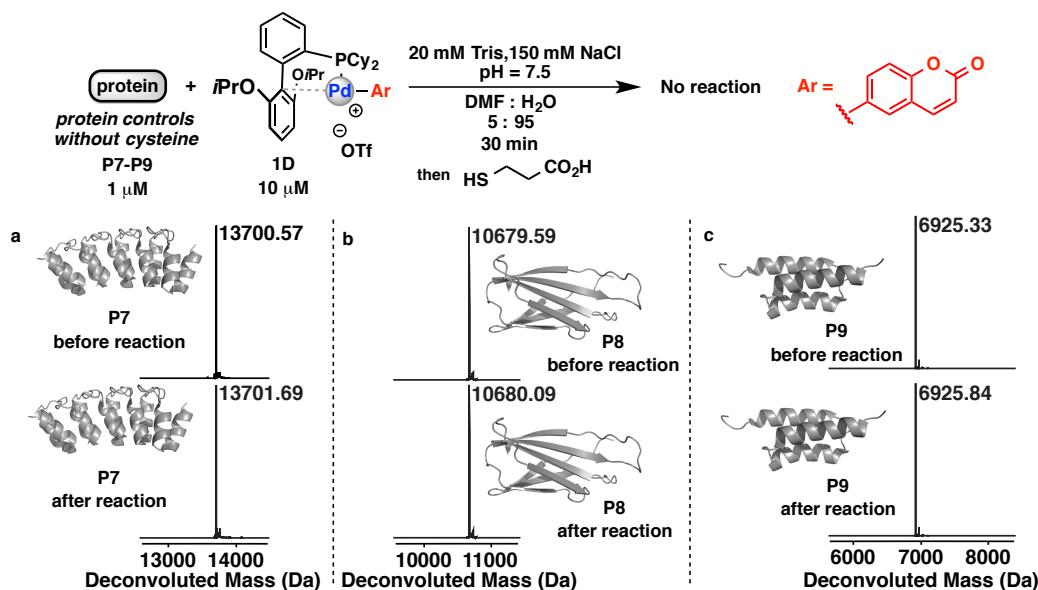
To a solution of protein (500 pmoles) in 475  $\mu$ L of 20 mM Tris and 150 mM NaCl buffer (pH 7.5) was added palladium-coumarin complex **1D** or palladium-drug complex **1J** (25  $\mu$ L, 200  $\mu$ M) in DMF. The solution was pipetted up and down 20 times to ensure proper reagent mixing. The reaction mixture was left at room temperature for 30 min. After this time, the reaction was quenched by the addition of 3-mercaptopropionic acid (25  $\mu$ L, 2 mM) dissolved in 20 mM Tris and 150 mM NaCl buffer (pH 7.5). After an additional 5 min at rt, 500  $\mu$ L of 1 : 1  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (v/v) containing 0.2% TFA was added and the resulting mixture was analyzed by LC-MS.

**Table S6. Protein sequences and calculated masses**

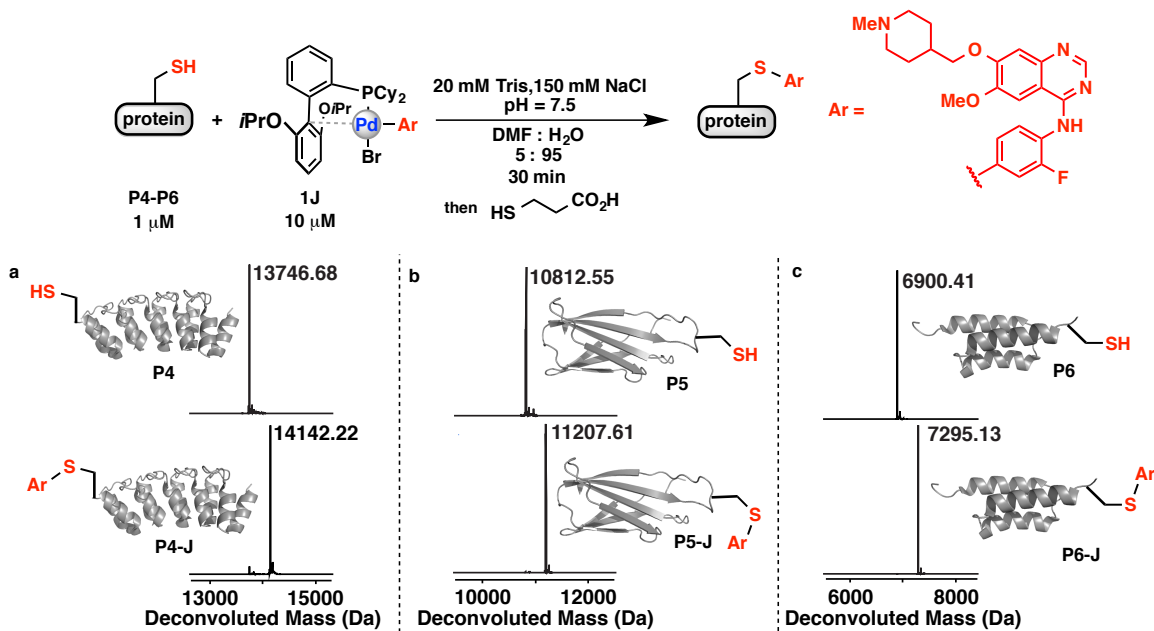
<b>Protein P4: DARPin-Cys</b>	<b>Calculated Mass: 13747.3 Da</b>
<b>Sequence<sup>a</sup>:</b>	
GGGGGSDLGKKLLEAARAGQDDEVRLMANGADVNA YDDNGVTPLHLAAFLGHLEI VEVLLKYGADVNAADSWGTTPLHLAATWGHLEIVEVLLKHGADVNAQDKFGKTAF DISIDNGNEDLAEILQKLN	
<b>Protein P7: DARPin</b>	<b>Calculated Mass: 13701.3 Da</b>
<b>Sequence:</b>	
GGGGGSDLGKKLLEAARAGQDDEVRLMANGADVNA YDDNGVTPLHLAAFLGHLEI VEVLLKYGADVNAADSWGTTPLHLAATWGHLEIVEVLLKHGADVNAQDKFGKTAF DISIDNGNEDLAEILQKLN	
<b>Protein P5: 10FN3-Cys</b>	<b>Calculated Mass: 10813.1 Da</b>
<b>Sequence:</b>	
SVSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTAT ISGLKPGVDYTITVYAVTLPSTCGASSKPISINYRTEIDKPSQ	
<b>Protein P8: 10FN3</b>	<b>Calculated Mass: 10679.9 Da</b>
<b>Sequence:</b>	
VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATI SGLKPGVDYTITVYAVTLPSTGGASSKPISINYRTEIDKPSQ	
<b>Protein P6: Affibody-Cys</b>	<b>Calculated Mass: 6900.6</b>
<b>Sequence:</b>	
GGGGGVVDNKFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEAKKLN DA <sup>a</sup> CPK	
<b>Protein P9: Affibody</b>	<b>Calculated Mass: 6925.6 Da</b>
<b>Sequence:</b>	
GGGGGVVDNKFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEAKKLN DAQAPK	

<sup>a</sup> Cysteine residues are highlighted in red.

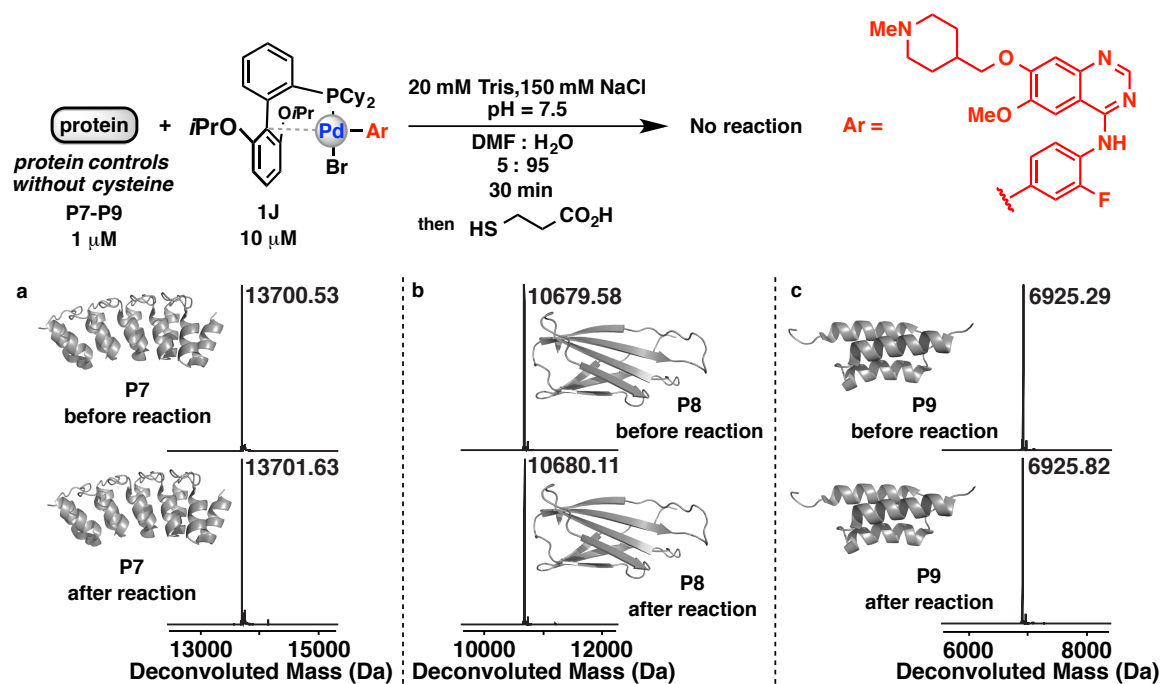
**Figure S7.** Control reactions for protein labeling with palladium complex **1D** (reactions with proteins without cysteine residues).



**Figure S8.** Protein labeling with palladium complex **1J**.

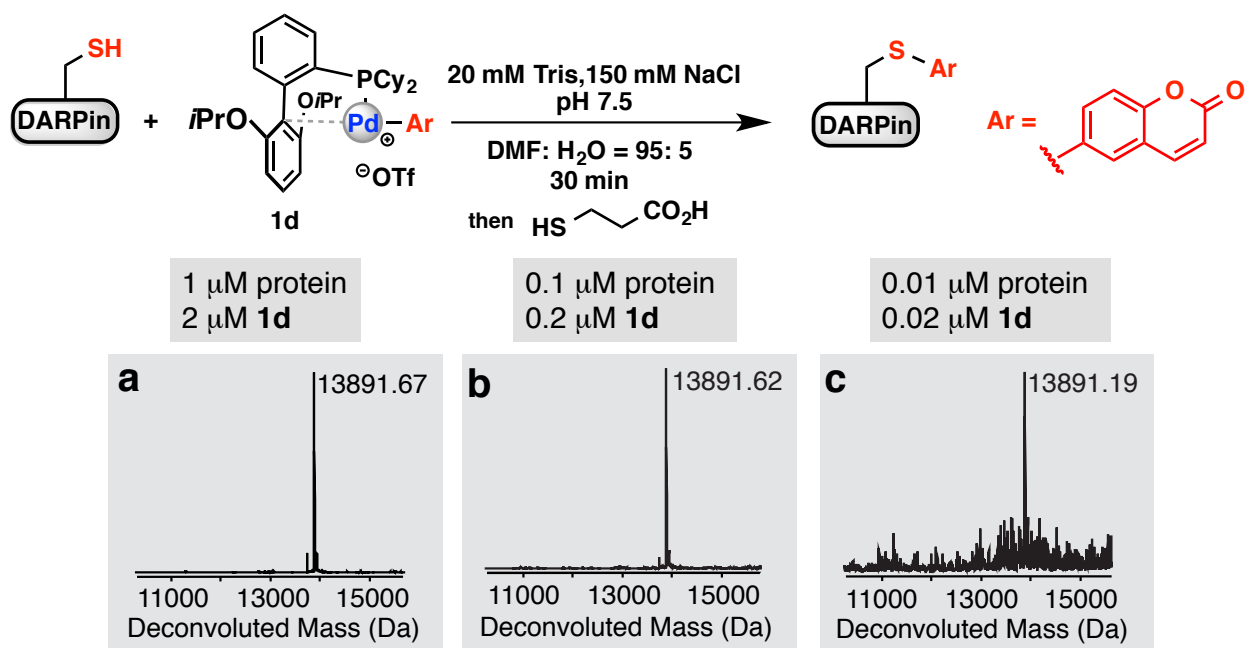


**Figure S9.** Control reactions for protein labeling with palladium complex **1J** (reactions with proteins without cysteine residues).



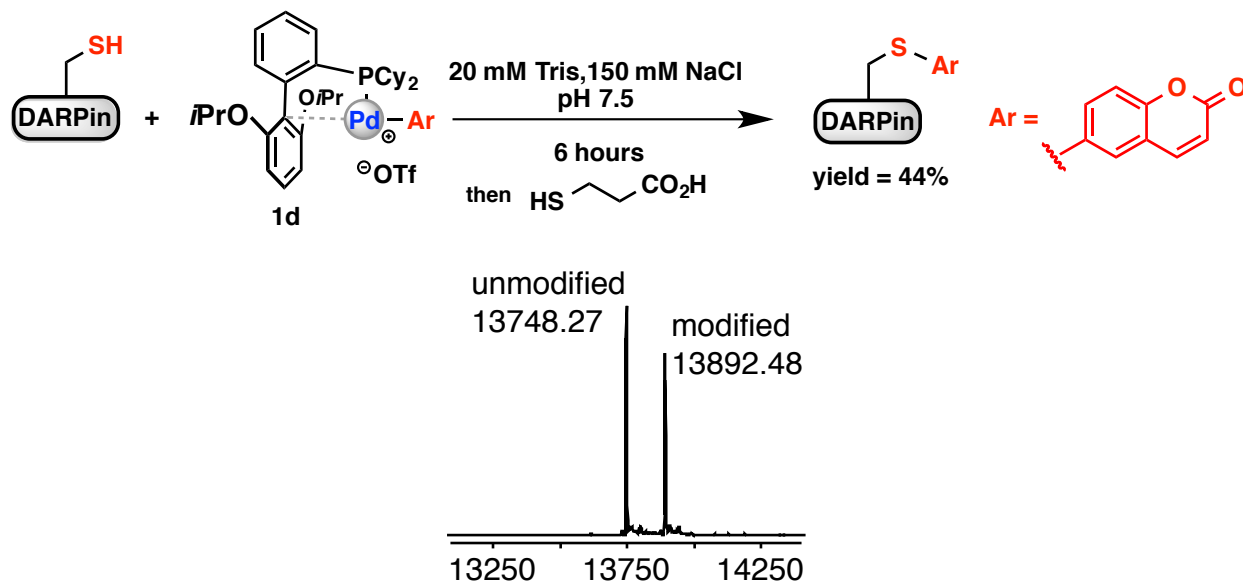
### Reactions at low protein concentrations

**Figure S10.** Protein labeling experiments at low concentrations.



### Reaction without organic solvent

**Figure S11.** Protein labeling experiments without organic solvent.



### Protein Activity Experiments

#### Synthesis and purification of modified LF<sub>N</sub>-DTA variants

Lethal factor *N*-terminal domain fused to diphtheria toxin A-chain (LF<sub>N</sub>-DTA) was expressed and purified as described before.<sup>14</sup> A C-terminal LPSTGGH<sub>6</sub> tag was included for the Sortase-mediated peptide conjugation. Cysteine-containing peptide NH<sub>2</sub>-GGGGGLRLCA-C(O)NH<sub>2</sub> and its serine variant NH<sub>2</sub>-GGGGGLRLSA-C(O)NH<sub>2</sub> were synthesized, purified, and conjugated to the LF<sub>N</sub>-DTA-LPSTGGH<sub>6</sub> tag following the previously described protocol (Fig. S12).<sup>14</sup> Sequences for the resulting proteins **LF<sub>N</sub>-DTA-Cys** and **LF<sub>N</sub>-DTA-Ser** are shown below.

#### LF<sub>N</sub>-DTA-Cys

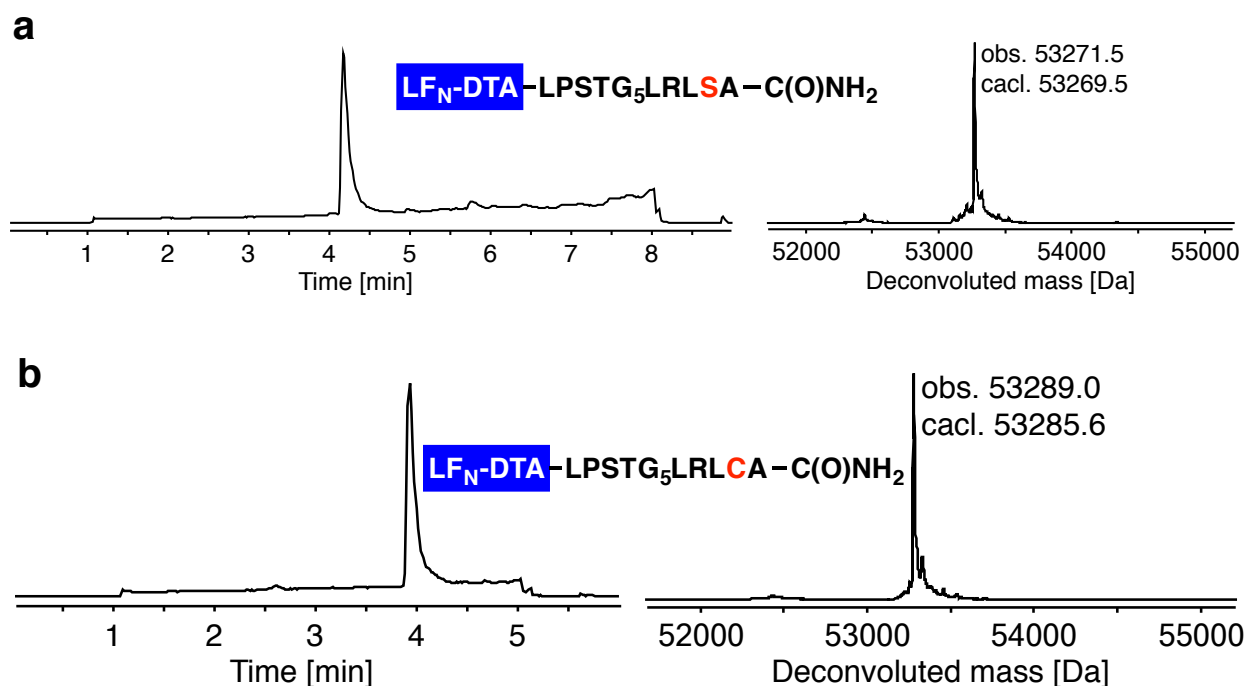
AGGHGDVGMHVKEKEKNKDENKRKDEERNKTQEEHLKEIMKHIVKIEVKGEEAVKKE  
AAEKLLEKVPDVLVEMYKAIGGKIYIVDGDITKHISLEALSEDKKKIKDIYGKDALLHEH  
YVYAKEGYEPVLVIQSSDYVENTEKALNVYYEIGKILSRDILSKINQPYQKFLDVLNTIK  
NASDSGQDLLFTNQLKEHPTDFSVEFLEQNSNEVQEVFAKAFAYYIEPQHRDVLQLYA  
PEAFNYMDKFNEQEINLSLEELKDQRSGRELERGADDVVDSSKSFVMENFSSYHGTPG  
YVDSIQKGIQKPKSGTQGNYYDDDWKGFYSTDNKYDAAGYSVDNENPLSGKAGGVVKV  
TYPGLTKVLALKVDNAETIKKELGLSLTEPLMEQVGTEEFIKRFGDGASRVVLSLPFAEG

SSSVEYINNWEQAKALSVELEINFETRGRGQDAMYEYMAQASAGNRLPSTGGGGGLR  
 LCA-C(O)NH<sub>2</sub>

### LF<sub>N</sub>-DTA-Ser

AGGHGDVGMHVKEKEKNKDENKRKDEERNKTQEEHLKEIMKHIVKIEVKGEEAVKKE  
 AAELLEKVPSPDVLEMYKAIGGKIYIVDGDITKHISLEALSEDKKKIKDIYGKDALLHEH  
 YVYAKEGYEPVLVIQSSSEDYVENTEKALNVYYEIGKILSRDILSKINQPYQKFLDVLNTIK  
 NASDSGDQDLLFTNQLKEHPTDFSVEFLEQNSNEVQEVFAKAFAYYIEPQHRDVLQLYA  
 PEAFFNYMDKFNQEINLSLEELKDQRSGRELERGADDVVDSSKSFVMENFSSYHGTPG  
 YVDSIQKGIQPKSGTQGNYYDDDWKGFYSTDNKYDAAGYSVDNENPLSGKAGGVVKV  
 TYPGLTKVLALKVDNAETIKKELGLSLTEPLMEQVGTEEFIKRFGDGASRVVLSLPFAEG  
 SSSVEYINNWEQAKALSVELEINFETRGRGQDAMYEYMAQASAGNRLPSTGGGGGLR  
 LSA-C(O)NH<sub>2</sub>

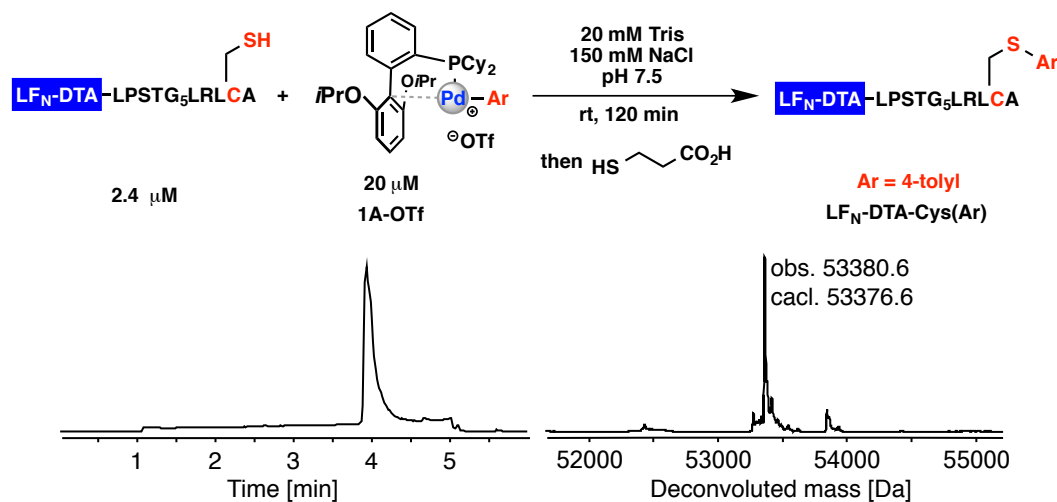
**Figure S12.** LC-MS analysis of the purified LF<sub>N</sub>-DTA-Ser (a) and LF<sub>N</sub>-DTA-Cys (b). Total ion current chromatograms (left) and the deconvoluted mass spectra (right) of the full protein peaks are shown.



### Arylation of **LF<sub>N</sub>-DTA-Cys** using **1A-OTf**

Complex **1A-OTf** (100  $\mu$ L; 400  $\mu$ M in DMF) was added to a solution of **LF<sub>N</sub>-DTA-Cys** (2.5  $\mu$ M, 1.9 mL) in buffer P (20 mM Tris, 150 mM NaCl, pH 7.5). The final reaction conditions are: 2.4  $\mu$ M of **LF<sub>N</sub>-DTA-Cys**, 20  $\mu$ M of **1A-OTf**, 150 mM NaCl, 20 mM Tris, pH 7.5, 5% DMF. The reaction mixture was pipetted up and down 20 times to afford proper mixing and was rotated at room temperature for 2 h, after which 20  $\mu$ L of 20 mM thiopropionic acid in buffer P (10 equivalents of thiopropionic acid relative to the palladium reagent) was added to quench the reaction. The reaction mixture was incubated at room temperature for additional 5 min and was then subjected to purification by size-exclusion chromatography (SEC) using buffer P (20 mM Tris, 150 mM NaCl, pH 7.5) as the elution buffer. The fractions were analyzed by gel electrophoresis and the fractions containing the desired protein product were combined, concentrated, and buffer exchanged into phosphate buffer saline (PBS) using 10K spin concentrator (EMD Millipore). The resulting modified protein **LF<sub>N</sub>-DTA-Cys(Ar)** was recovered in 56% yield as measured by UV280. This procedure allowed for the removal of 91% of the palladium species in the crude reaction mixture as measured by ICP-MS (see the ICP-MS section for details).

**Figure S13.** LC-MS analysis of the SEC-purified **LF<sub>N</sub>-DTA-Cys(Ar)**. Total ion current chromatogram (right) and the deconvoluted mass spectrum of the full protein peak (left) are shown.

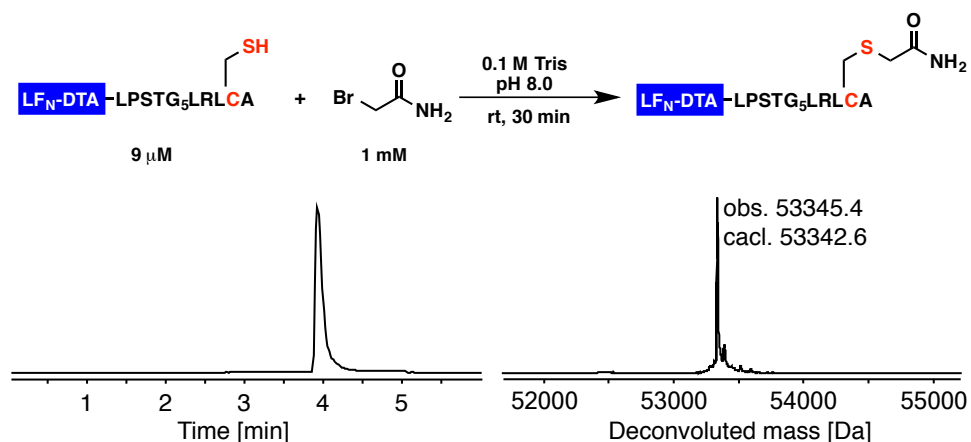


### Alkylation of **LF<sub>N</sub>-DTA-Cys**

Tris buffer (60  $\mu$ L, 1M, pH 8.0) and bromoacetamide (60  $\mu$ L, 10 mM in water) were added to a solution of **LF<sub>N</sub>-DTA-Cys** (11  $\mu$ M, 480  $\mu$ L) in buffer P. The final reaction conditions

are: 9  $\mu$ M LF<sub>N</sub>-DTA-Cys, 1 mM bromoacetamide, 0.1 M Tris, pH 8.0. The reaction mixture was pipetted up and down 20 times to afford proper mixing and was left at room temperature for 30 minutes. Upon completion, the reaction mixture was buffer exchanged 5 times with PBS buffer using 10K spin concentrator (EMD Millipore). The purified protein is denoted as LF<sub>N</sub>-DTA-Cys(Alk).

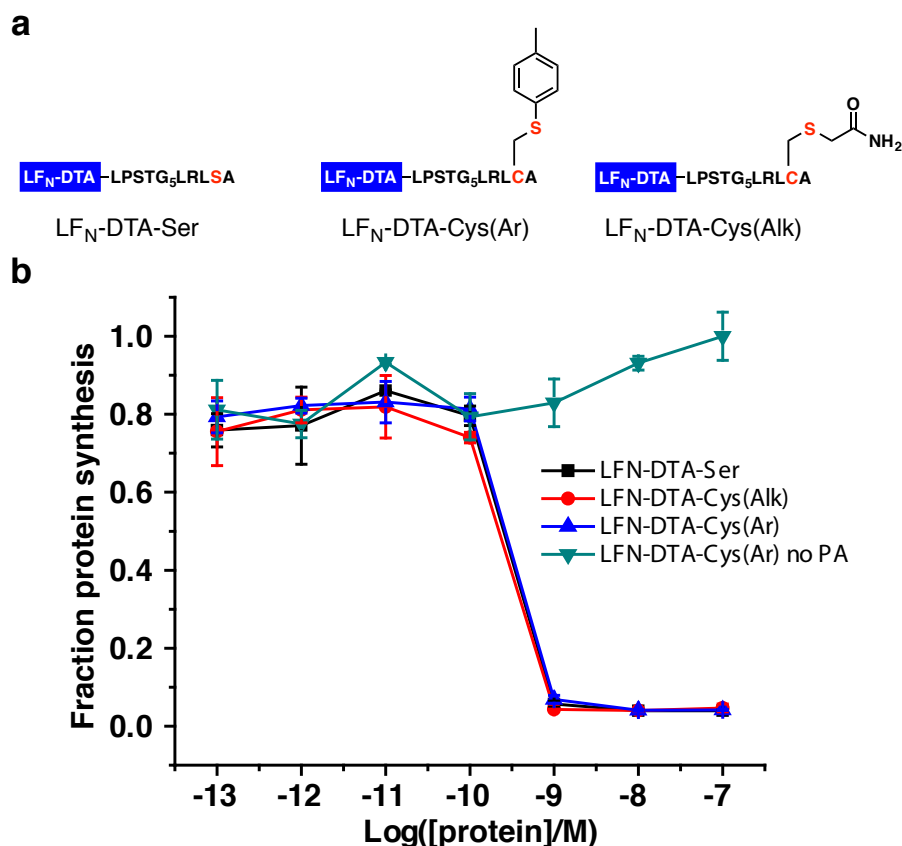
**Figure S14.** Alkylation of the LF<sub>N</sub>-DTA-Cys and LC-MS analysis of the purified LF<sub>N</sub>-DTA-Cys(Alk). Total ion current chromatogram (left) and the deconvoluted mass spectrum of the full protein peak (right) are shown.



### Protein synthesis inhibition assay

The translocation assay was performed as described previously.<sup>14</sup> The CHO-K1 cells were maintained in the F-12K media supplemented with 10% (v/v) fetal bovine serum at 37 °C and 5% CO<sub>2</sub> in humidified atmosphere. The cells were plated at  $3.0 \times 10^4$ /well in a 96-well white opaque plate one day prior to the assay. Modified LF<sub>N</sub>-DTA variants (Figure S14a) were prepared in ten-fold serial dilutions in 50  $\mu$ L of F-12K media each, after which additional 50  $\mu$ L of F-12K media containing 20 nM of protective antigen (PA) were added. The resulting 100  $\mu$ L samples were added to the CHO-K1 cells and incubated for 2 hours at 37 °C and 5% CO<sub>2</sub>. The cells were then washed three times with PBS, after which 100  $\mu$ L of leucine-free F-12K medium supplemented with 1  $\mu$ Ci/mL <sup>3</sup>H-leucine (Perkin Elmer, MA) was added. The resulting mixture was incubated for an additional 1 h at 37 °C with 5% CO<sub>2</sub>. The cells were washed three times with PBS and suspended in 150  $\mu$ L of scintillation fluid. <sup>3</sup>H-Leu incorporation into cellular proteins was measured to determine the inhibition of protein synthesis by the LF<sub>N</sub>-DTA variants. Scintillation counts from cells treated with only PA were used as control values for normalization. Each experiment was done in triplicate.

**Figure S15.** Translocation efficiencies of the LF<sub>N</sub>-DTA variants were analyzed by protein synthesis inhibition assay. LF<sub>N</sub>-DTA-Cys(Ar) showed similar protein synthesis inhibition profile as the controls (LF<sub>N</sub>-DTA-Cys(Alk) and LF<sub>N</sub>-DTA-Ser), indicating that the palladium-mediated bioconjugation and the following purification procedure did not significantly alter the activity of the protein. EC<sub>50</sub> values for each protein are: 0.40 ± 0.09 nM for LF<sub>N</sub>-DTA-Cys(Ar), 0.20 ± 0.01 nM for LF<sub>N</sub>-DTA-Cys(Alk), and 0.25 ± 0.05 nM for LF<sub>N</sub>-DTA-Ser. EC<sub>50</sub> values were obtained by fitting the curves to sigmoidal Boltzmann equation using OriginLab 8.0 software (Northhampton, MA).



## 8. Antibody Labeling Experiments

### Expression and Purification of Trastuzumab

Trastuzumab was expressed from the gWiz-trastuzumab plasmids. The light chain and heavy chain sequences for trastuzumab used in this study are:

#### *Trastuzumab-Light Chain*

DIQMTQSPSSLSASVGDRVITICRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVP  
SRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTRTVAAPSVFIFP

PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST  
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

### ***Trastuzumab-Heavy Chain***

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG  
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQ  
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT  
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC  
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK  
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ  
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLY  
SKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

The IgGs were expressed via transient transfections of HEK293F cells (Invitrogen) as previously described,<sup>15</sup> and purified using Protein A affinity chromatography (Genscript) following manufacturer's instructions. The purified IgGs were analyzed by LC-MS to confirm their molecular weight and purity, and stored in PBS at -80 °C.

### **Synthesis and purification of an Antibody-Drug Conjugate (ADC).**

TCEP (1 µL, 25 mM in water) and Tris buffer (5 µL, 1M, pH 8.0) were added to a solution of trastuzumab (57 µM, 44 µL) in PBS. The reaction mixture was pipetted up and down 20 times and incubated in a water bath at 37 °C for 2 h. The final reaction conditions for the reduction are: 50 µM antibody, 500 µM TCEP, 0.1 M Tris, pH 8.0.

Tris buffer (187.5 µL, 0.1 M, pH 8.0) and palladium-drug complex **1J** (12.5 µL, 2 mM in DMF) were added to the partially reduced antibody, the reaction mixture was pipetted up and down 20 times and was left at room temperature for 30 minutes. The final reaction conditions are: 10 µM antibody, 100 µM **1J**, 0.1 M Tris, pH 8.0, 5% DMF. The reaction mixture was purified using three protocols shown below to assess the protein recovery and palladium removal efficiency in each protocol.

### ***Purification protocol 1***

The crude reaction mixture was directly loaded on a size-exclusion chromatography (SEC) column (GE Healthcare) for purification using buffer P (20 mM Tris, 150 mM NaCl, pH 7.5) as

the elution buffer. The fractions were collected and analyzed by gel electrophoresis. The fractions containing antibodies were then combined, concentrated, and buffer exchanged into phosphate buffer saline (PBS) using 10K spin concentrator (EMD Millipore). The antibody was recovered in 47% yield as measured by UV280. This purification protocol allowed for the removal of 81% of the palladium species from the crude reaction mixture as determined by ICP-MS (see details in the ICP-MS section). This protein sample was used in the Octet BioLayer Interferometry binding assay (see Fig. S16 in the Octet BioLayer Interferometry binding assay section).

### ***Purification protocol 2***

The crude reaction mixture was quenched with a solution of thiopropionic acid (0.5  $\mu$ L, 50 mM in buffer P, 10 equivalents relative to the amount of the palladium reagent used), and the resulting solution was incubated at room temperature for 5 minutes. The solution was filtered through a 0.2  $\mu$ m nylon spin filter (PALL Life Sciences). The filtered solution was loaded on a PD-10 desalting column (GE Healthcare) pre-equilibrated with TPA buffer (20 mM Tris, 150 mM NaCl, 1 mM thiopropionic acid). The antibody was eluted into 3.5 mL of TPA buffer following the manufacturer's protocol. The resulting solution was buffer exchanged into phosphate buffer saline (PBS) using 10K spin concentrator (EMD Millipore). The antibody was recovered in 86% yield as measured by UV280. This purification procedure allowed for removal of 73% of the palladium species from the crude reaction mixture as determined by ICP-MS (see details in the ICP-MS section).

### ***Purification protocol 3***

The crude reaction mixture was quenched with a solution of thiopropionic acid (0.5  $\mu$ L, 50 mM in buffer P, 10 equivalents relative to the amount of the palladium reagent used), and the resulting solution was incubated at room temperature for 5 minutes. The solution was filtered through a 0.2  $\mu$ m nylon spin filter (PALL Life Sciences). The filtered solution was loaded on a size-exclusion chromatography (SEC) column (GE Healthcare) pre-equilibrated with TPA buffer (20 mM Tris, 150 mM NaCl, 1 mM thiopropionic acid). The antibody was purified using TPA buffer as the elution buffer. The fractions were collected and analyzed by gel electrophoresis, and the fractions containing antibodies were combined, concentrated, and buffer exchanged into phosphate buffer saline (PBS) using 10K spin concentrator (EMD Millipore). The antibody was recovered in 39% yield as measured by UV280. The purification procedure allowed for the

removal of 94% of the palladium species from the crude reaction mixture as determined by ICP-MS (see details in the ICP-MS section).

### ***LC-MS analysis of the purified ADCs***

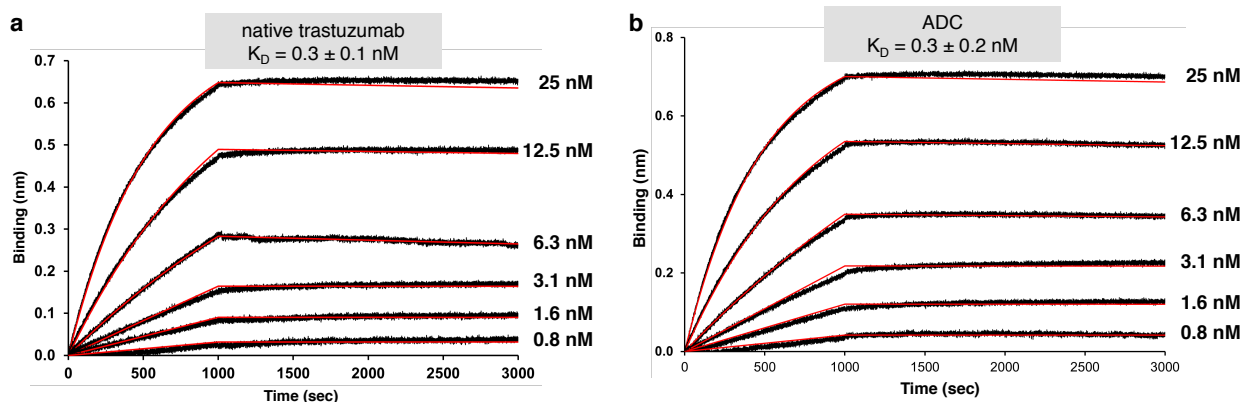
N-linked glycans were removed by the addition of 1  $\mu$ L of PNGase F (New England BioLabs) to the antibody (100  $\mu$ g in 100  $\mu$ L 0.1 M Tris, pH 8.0) and incubation of the mixture for 1 h at 45 °C. The resulting solution was reduced by the addition of 1/10 volume of 200 mM TCEP solution (pH 8.0) and incubation for 30 min at 37 °C. The resulting mixture was quenched by the addition of 10/1 volume of 50% A : 50% B (v/v) and analyzed by LC-MS. The ADC purified using *protocol 1* showed the drug-to-antibody ratio of 4.4.

### **Octet BioLayer Interferometry binding assay.**

The purified ADC and the native trastuzumab were biotinylated using EZ-Link NHS-(PEG)<sub>4</sub>-Biotin (Life Technologies). A solution of EZ-Link NHS-(PEG)<sub>4</sub>-Biotin (10  $\mu$ L, 200  $\mu$ M in PBS) was added to the corresponding protein (10  $\mu$ L, 20  $\mu$ M in PBS), after which the reaction mixture was pipetted up and down 20 times to allow proper mixing and was left at room temperature for 30 min. The crude reaction mixture was buffer exchanged with PBS for 5 times to remove the excess of NHS-(PEG)<sub>4</sub>-Biotin.

The biotinylated proteins were immobilized on streptavidin tips and were sampled with the serially diluted concentrations of recombinant HER2 (R&D Biosystems) in the Octet BioLayer Interferometry system. The obtained results were fitted according to the protocol provided by the manufacturer of the Octet BioLayer Interferometry system.

**Figure S16.** The ADC and the native trastuzumab showed similar binding affinity to HER2 in the Octet BioLayer Interferometry assay. Black curves are the experimental results, and red curves are the fitting results. The concentration of the recombinant HER2 used in each experiment is listed next to the corresponding curve.



## 9. ICP-MS

ICP-MS was used to measure the remaining palladium content in the purified LF<sub>N</sub>-DTA-Cys(Ar) protein and the purified antibody-drug conjugate (ADC). Protein samples were diluted in buffer solutions and lyophilized. The lyophilized powders were sent to Merck for ICP-MS analysis. The amount of remaining palladium in the purified sample was obtained from the calculations based on the measured palladium content in the ICP-MS samples. The palladium removal efficiency was reported as the percentage of palladium being removed from the sample compared to the palladium used in the crude reaction mixture. The obtained results are summarized in Table S7.

**Table S7.** Sample information for ICP-MS analysis of purified protein samples.

Protein Sample	Volume	Buffer	Concentration	Lyophilized Mass	Pd concentration
LF <sub>N</sub> -DTA-Cys(Ar)	0.1 mL	Buffer P	0.285 mg/mL	2.93 mg	26 ppm
ADC (Purification protocol 1)	0.5 mL	PBS	0.1 mg/mL	5.26 mg	28 ppm
ADC (Purification protocol 2)	0.5 mL	PBS	0.1 mg/mL	4.48 mg	25 ppm
ADC (Purification protocol 3)	0.5 mL	PBS	0.1 mg/mL	4.74 mg	12 ppm

### *Calculation of the palladium removal efficiency.*

#### ***LF<sub>N</sub>-DTA-Cys(Ar)***

100 µL of 400 µM of palladium complex **1A-OTf** was used in the crude reaction; this corresponds to the total palladium amount:

$$[\text{Total palladium}] = (100 \text{ }\mu\text{L}) * (400 \text{ }\mu\text{M}) * (106.42 \text{ g/mol}) = 4.26 \text{ }\mu\text{g}$$

56% of the protein was recovered, the total amount of protein recovered is:

$$[\text{Total protein recovered}] = (2.5 \text{ }\mu\text{M}) * (1.9 \text{ mL}) * (53286 \text{ g/mol}) * 0.56 = 0.14 \text{ mg}$$

Palladium concentration in the protein sample is:

$$[\text{Palladium concentration in protein}] = (26 \text{ ppm}) * (2.93 \text{ mg}) / ((0.1 \text{ mL}) * (0.285 \text{ mg/mL})) = 2673 \text{ ppm}$$

Palladium remaining in the purified protein is:

$$[\text{Palladium remaining}] = (2673 \text{ ppm}) * (0.14 \text{ mg}) = 0.37 \text{ }\mu\text{g}$$

Palladium removal efficiency is:

$$[\text{Palladium removal}] = 1 - [\text{Palladium remaining}] / [\text{Total palladium}] = 1 - (0.37 \text{ }\mu\text{g}) / (4.26 \text{ }\mu\text{g}) = 91\%$$

#### ***ADC (Protocol 1)***

12.5 µL of 2 mM of palladium complex **1J** was used in the crude reaction; this gives the total palladium amount in the crude reaction:

$$[\text{Total palladium}] = (12.5 \text{ }\mu\text{L}) * (2 \text{ mM}) * (106.42 \text{ g/mol}) = 2.66 \text{ }\mu\text{g}$$

86% of the protein was recovered, the total amount of protein recovered is:

$$[\text{Total protein recovered}] = (57 \text{ }\mu\text{M}) * (44 \text{ }\mu\text{L}) * (149 * 10^3 \text{ g/mol}) * 0.86 = 0.321 \text{ mg}$$

Palladium concentration in the protein sample is:

$$[\text{Palladium concentration in protein}] = (25 \text{ ppm}) * (4.48 \text{ mg}) / ((0.5 \text{ mL}) * (0.1 \text{ mg/mL})) = 2240 \text{ ppm}$$

Palladium remaining in the purified protein is:

$$[\text{Palladium remaining}] = (2240 \text{ ppm}) * (0.321 \text{ mg}) = 0.719 \text{ }\mu\text{g}$$

Palladium removal efficiency is:

$$[\text{Palladium removal}] = 1 - [\text{Palladium remaining}] / [\text{Total palladium}] = 1 - (0.719 \text{ }\mu\text{g}) / (2.66 \text{ }\mu\text{g}) = 73\%$$

### ***ADC (Protocol 2)***

12.5  $\mu\text{L}$  of 2 mM of palladium complex **1J** was used in the crude reaction; this gives the total palladium amount in the crude reaction:

$$[\text{Total palladium}] = (12.5 \mu\text{L}) * (2 \text{ mM}) * (106.42 \text{ g/mol}) = 2.66 \mu\text{g}$$

47% of the protein was recovered, the total amount of protein recovered is:

$$[\text{Total protein recovered}] = (57 \mu\text{M}) * (44 \mu\text{L}) * (149 * 10^3 \text{ g/mol}) * 0.47 = 0.175 \text{ mg}$$

Palladium concentration in the protein sample is:

$$[\text{Palladium concentration in protein}] = (28 \text{ ppm}) * (5.26 \text{ mg}) / ((0.5 \text{ mL}) * (0.1 \text{ mg/mL})) =$$

2946 ppm

Palladium remaining in the purified protein is:

$$[\text{Palladium remaining}] = (2946 \text{ ppm}) * (0.175 \text{ mg}) = 0.516 \mu\text{g}$$

Palladium removal efficiency is:

$$[\text{Palladium removal}] = 1 - [\text{Palladium remaining}] / [\text{Total palladium}] = 1 - (0.516 \mu\text{g}) / (2.66 \mu\text{g}) = 81\%$$

### ***ADC (Protocol 3)***

12.5  $\mu\text{L}$  of 2 mM of palladium complex **1J** was used in the crude reaction; this gives the total palladium amount in the crude reaction:

$$[\text{Total palladium}] = (12.5 \mu\text{L}) * (2 \text{ mM}) * (106.42 \text{ g/mol}) = 2.66 \mu\text{g}$$

39% of the protein was recovered, the total amount of protein recovered is:

$$[\text{Total protein recovered}] = (57 \mu\text{M}) * (44 \mu\text{L}) * (149 * 10^3 \text{ g/mol}) * 0.39 = 0.146 \text{ mg}$$

Palladium concentration in the protein sample is:

$$[\text{Palladium concentration in protein}] = (12 \text{ ppm}) * (4.74 \text{ mg}) / ((0.5 \text{ mL}) * (0.1 \text{ mg/mL})) =$$

1138 ppm

Palladium remaining in the purified protein is:

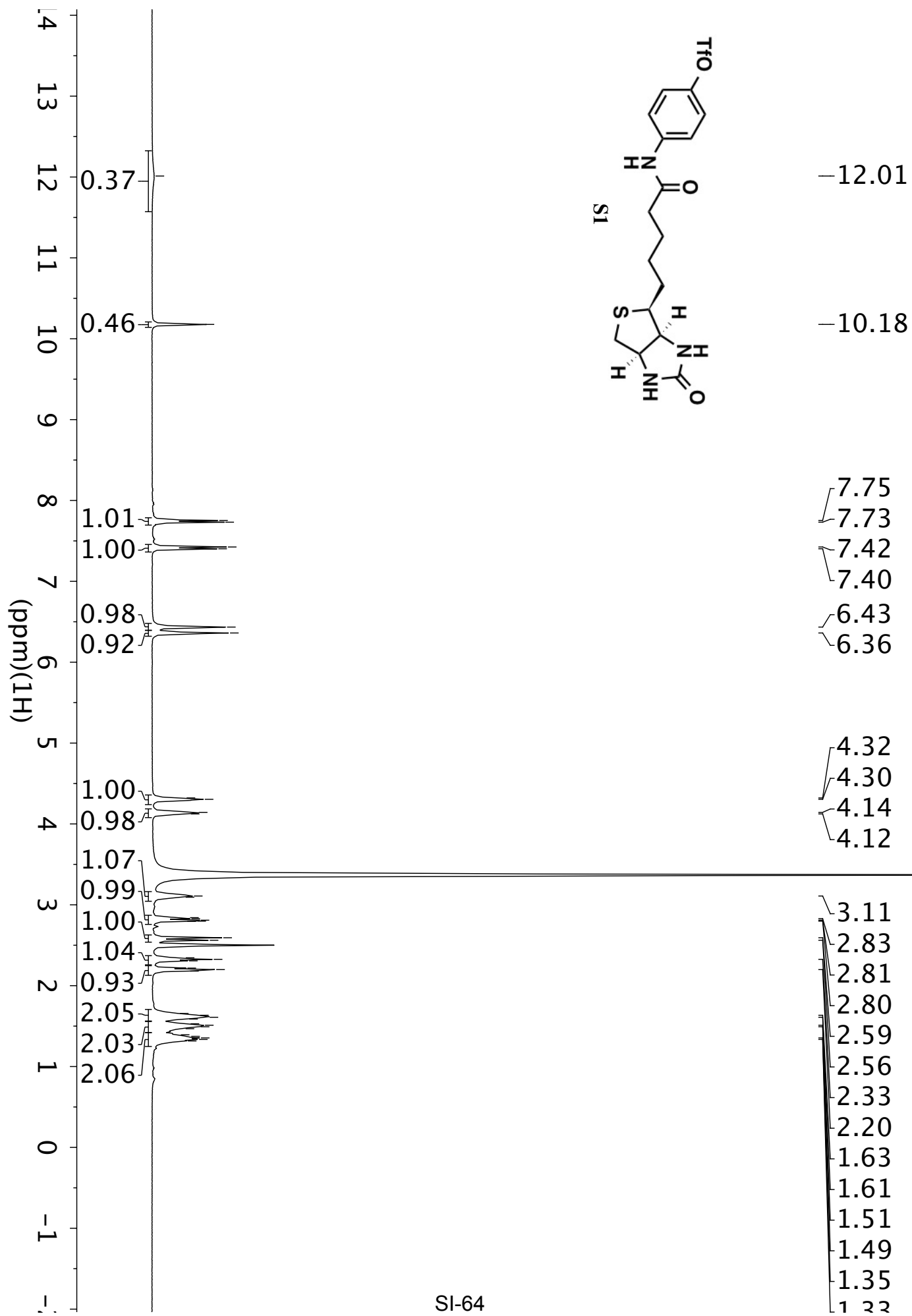
$$[\text{Palladium remaining}] = (1138 \text{ ppm}) * (0.146 \text{ mg}) = 0.166 \mu\text{g}$$

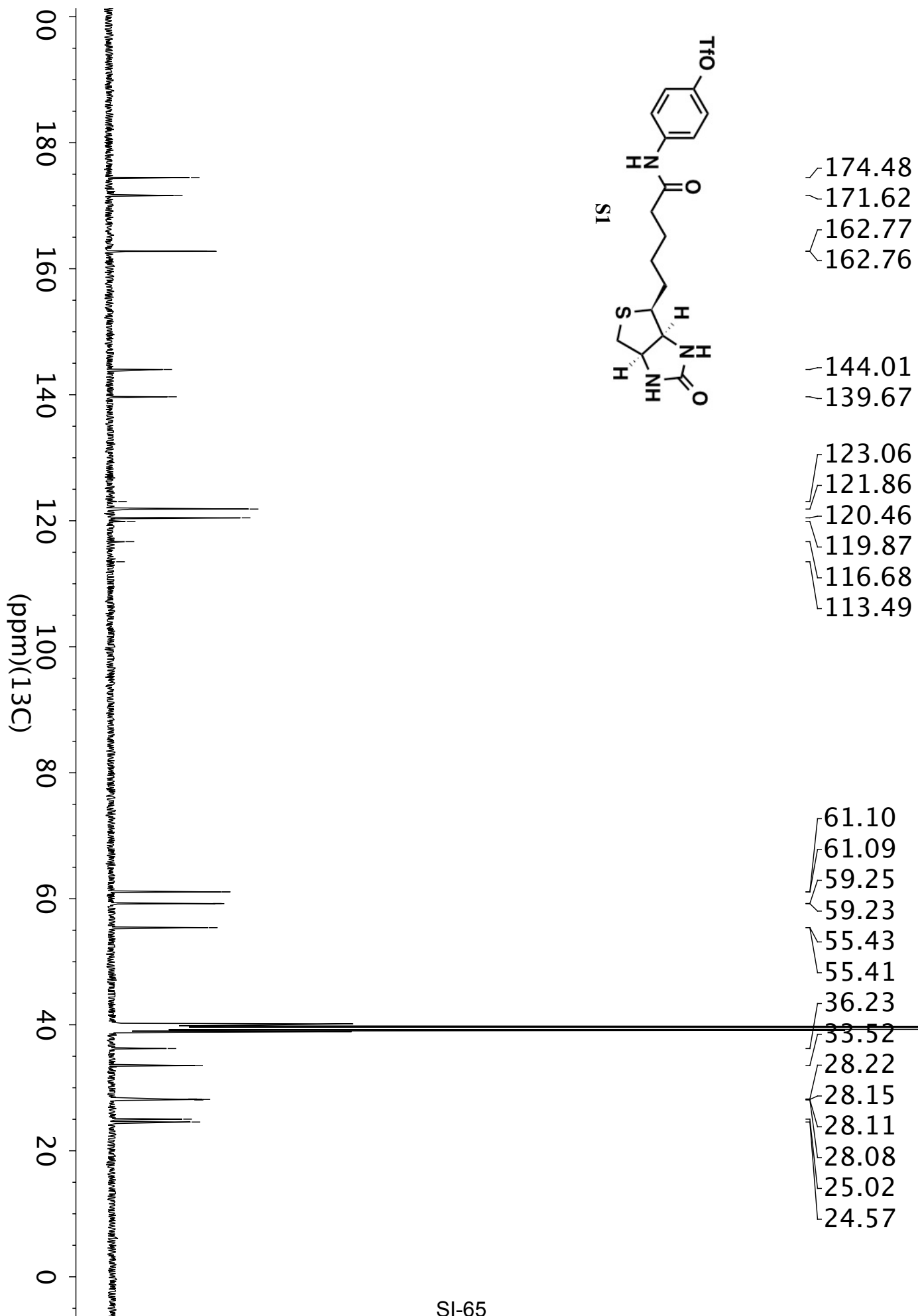
Palladium removal efficiency is:

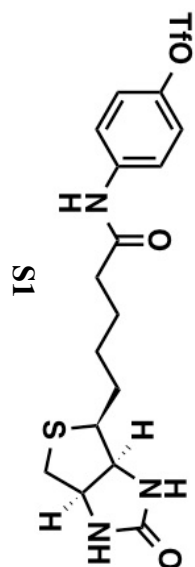
$$[\text{Palladium removal}] = 1 - [\text{Palladium remaining}] / [\text{Total palladium}] = 1 - (0.166 \mu\text{g}) / (2.66 \mu\text{g}) = 94\%$$

## 10. References.

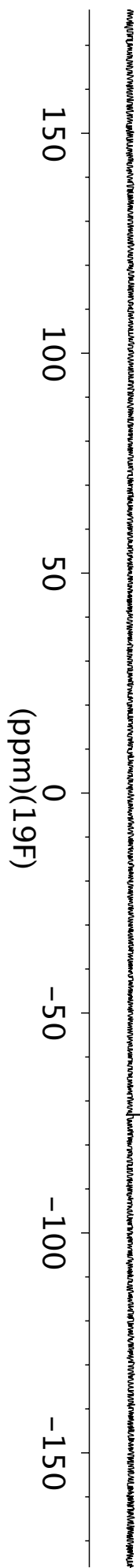
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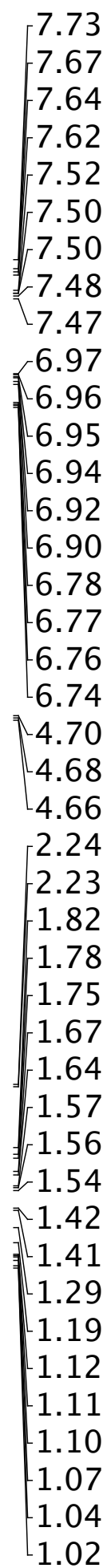
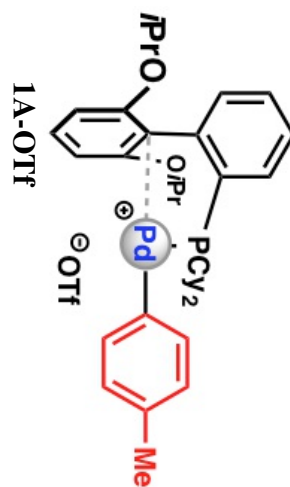
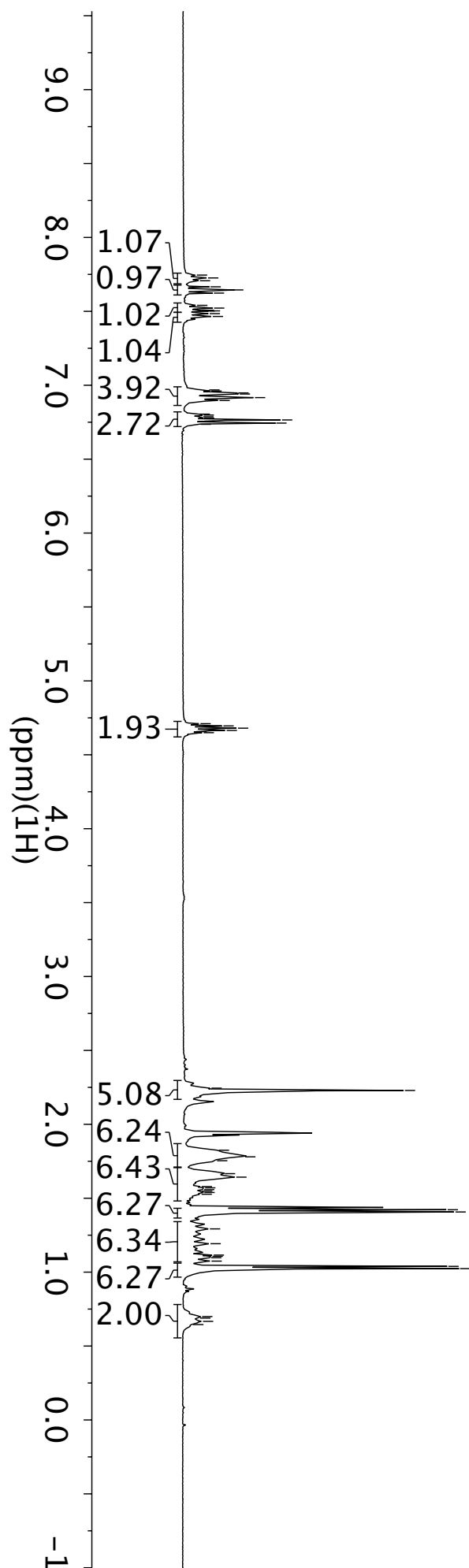


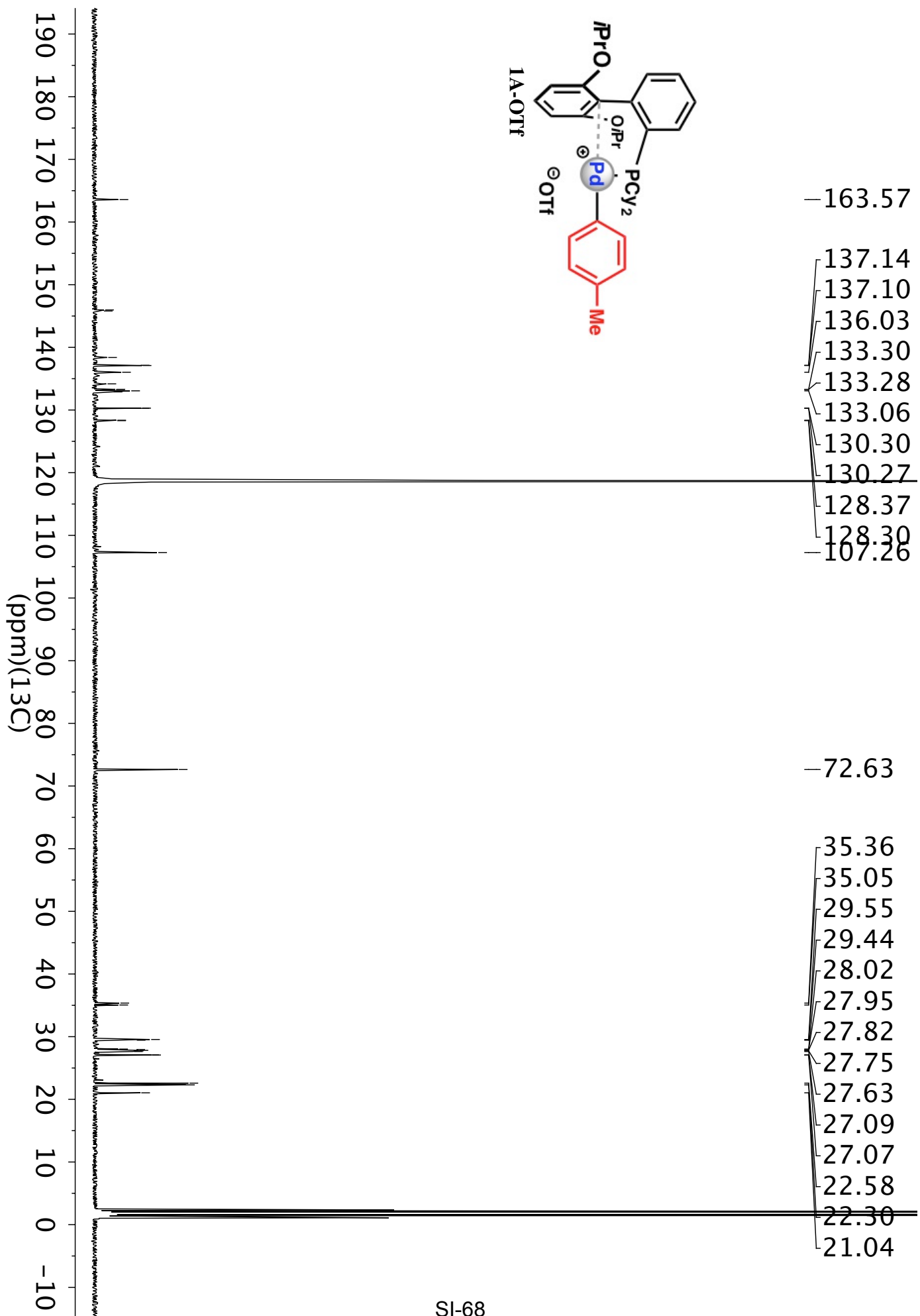




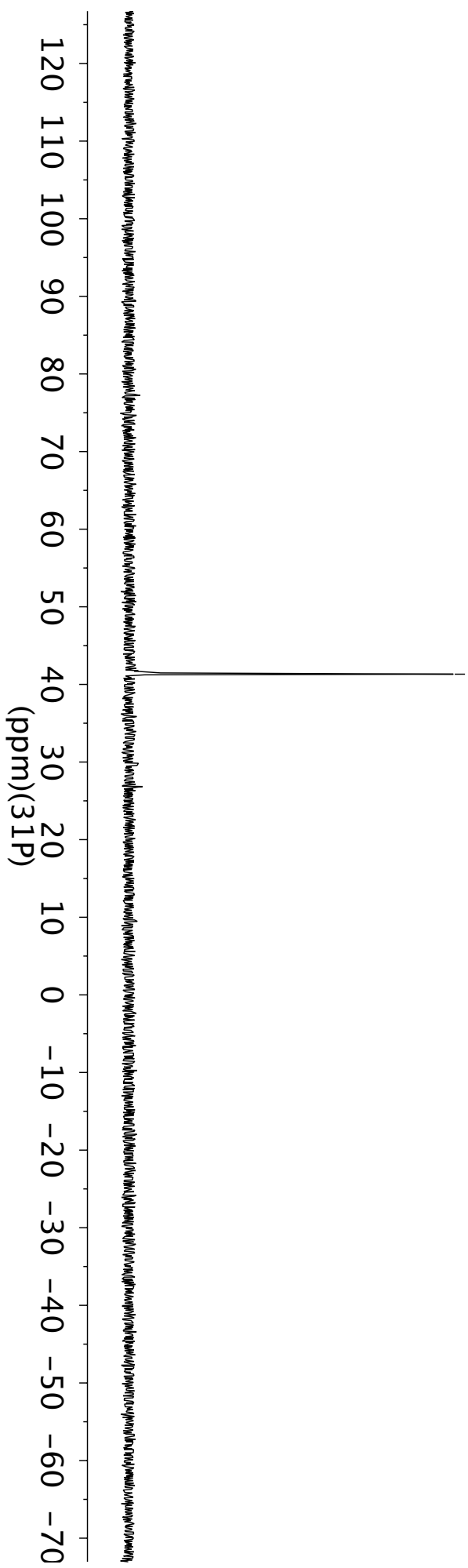
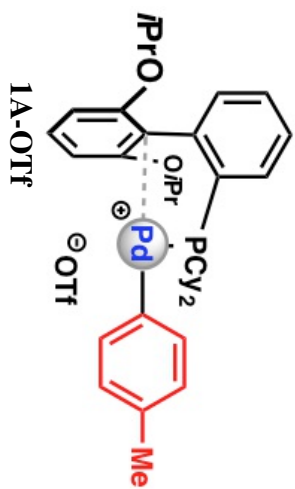
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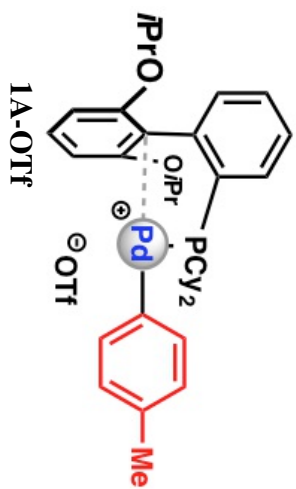




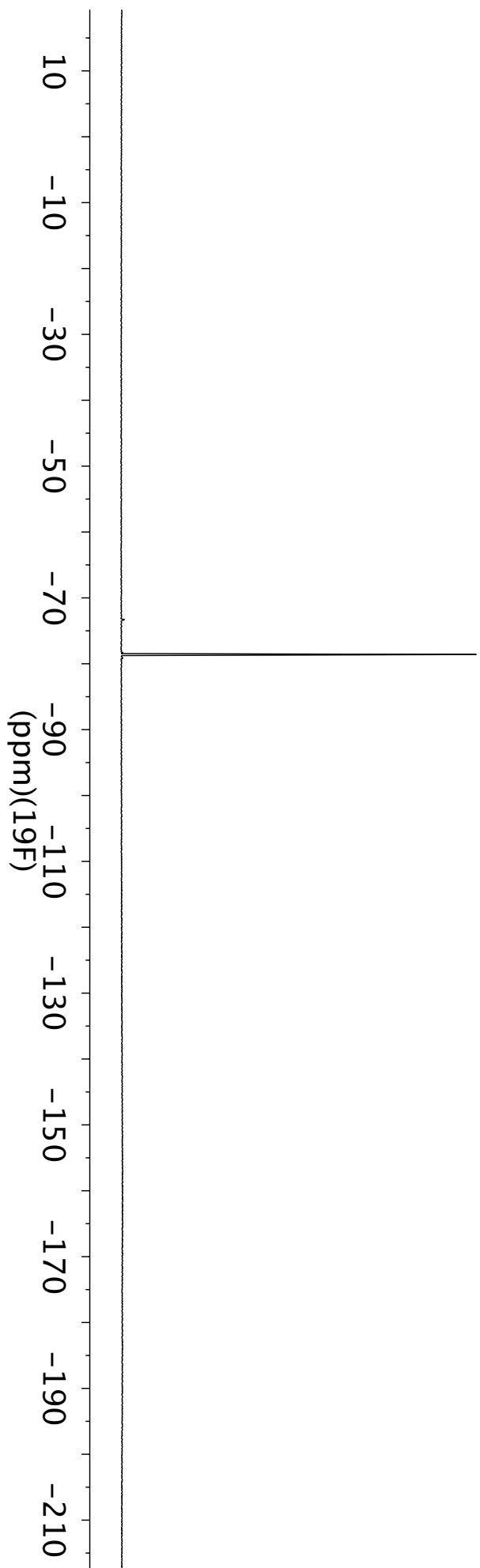


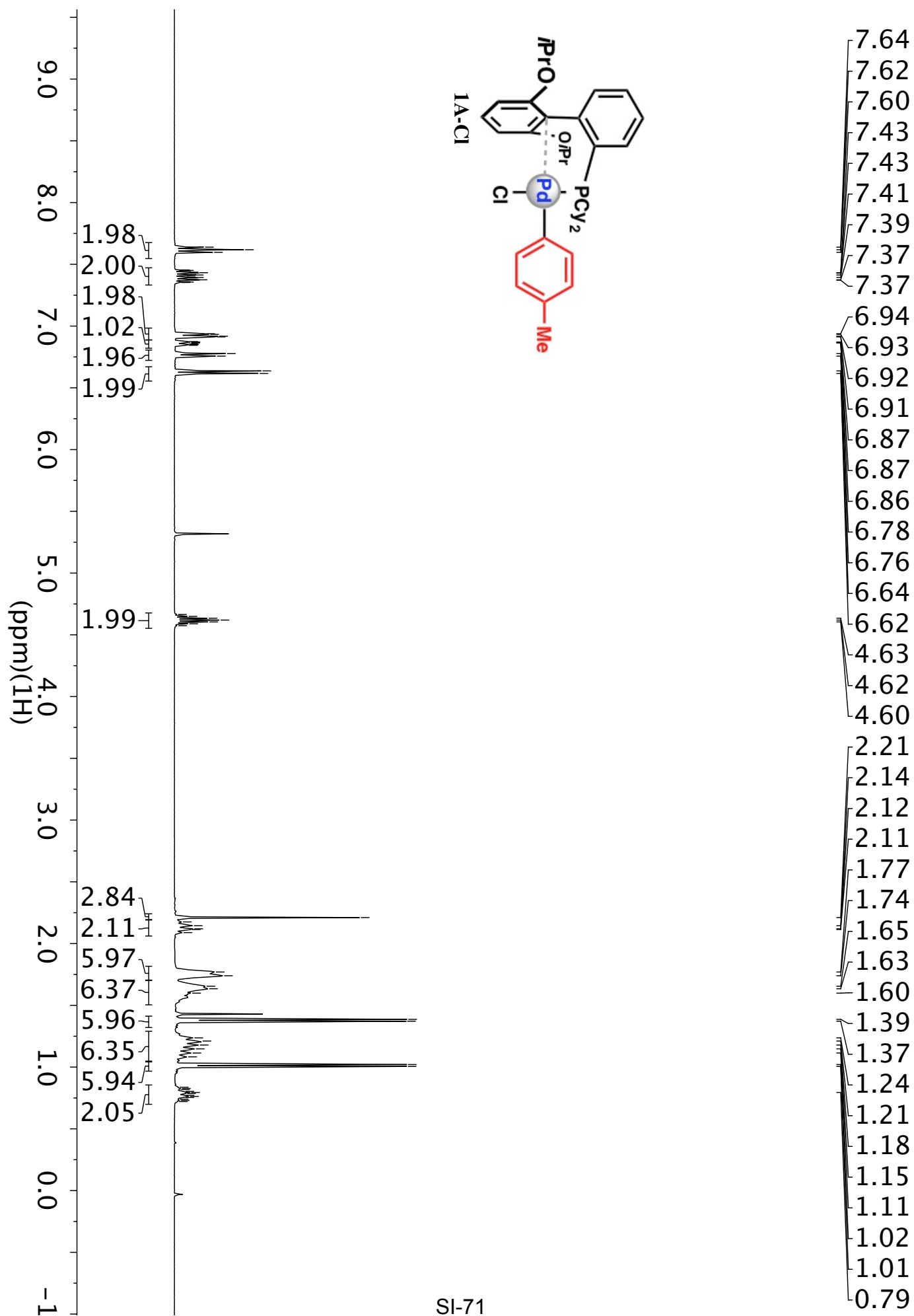
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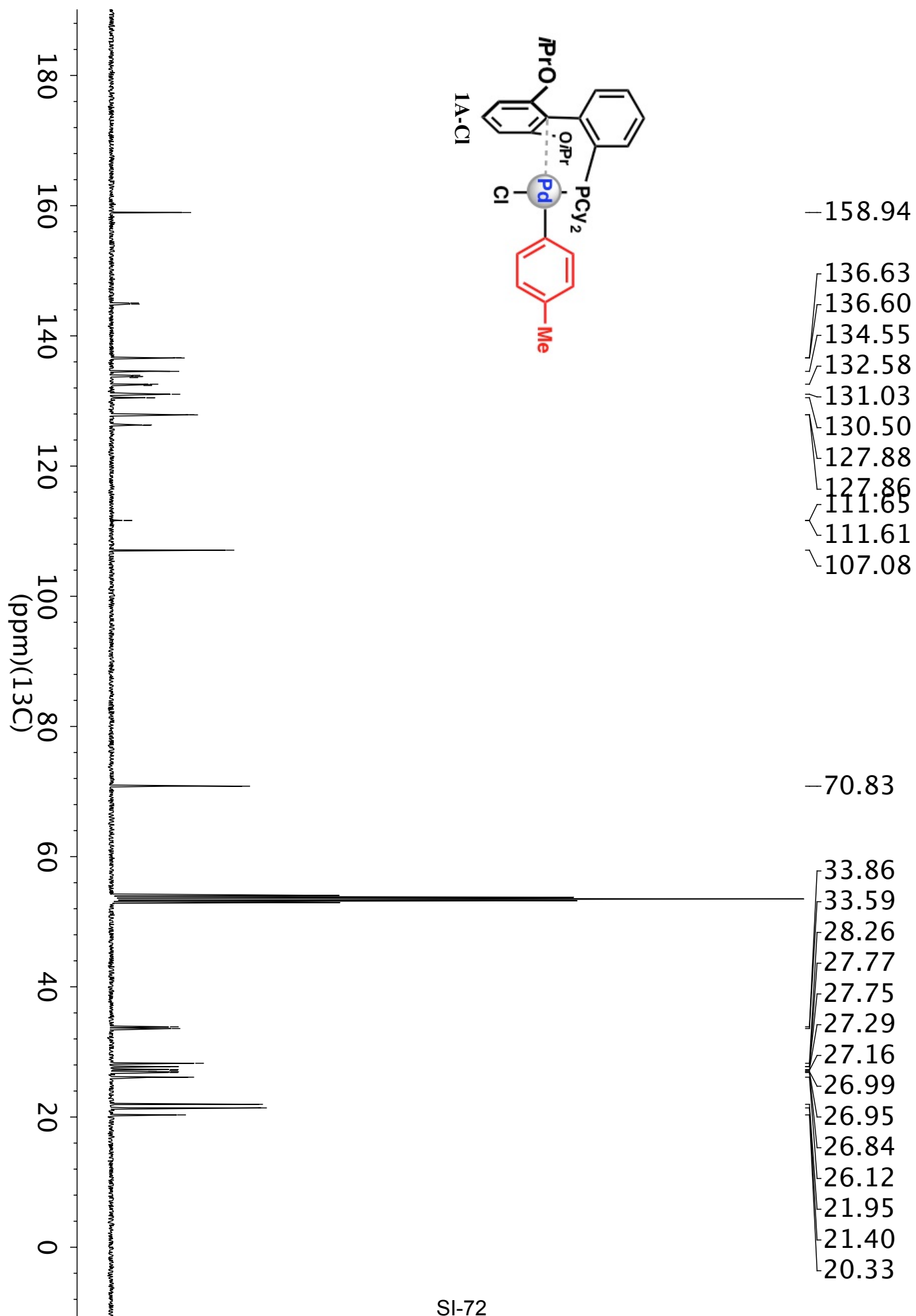




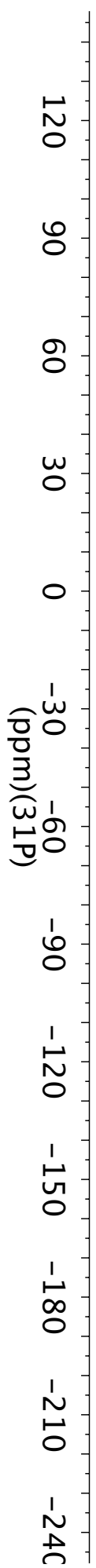
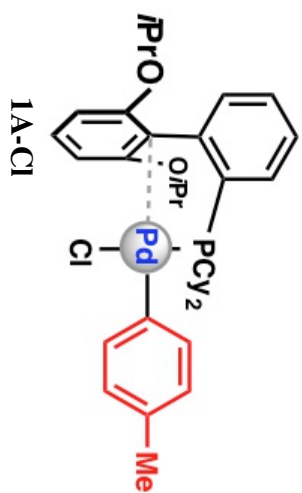
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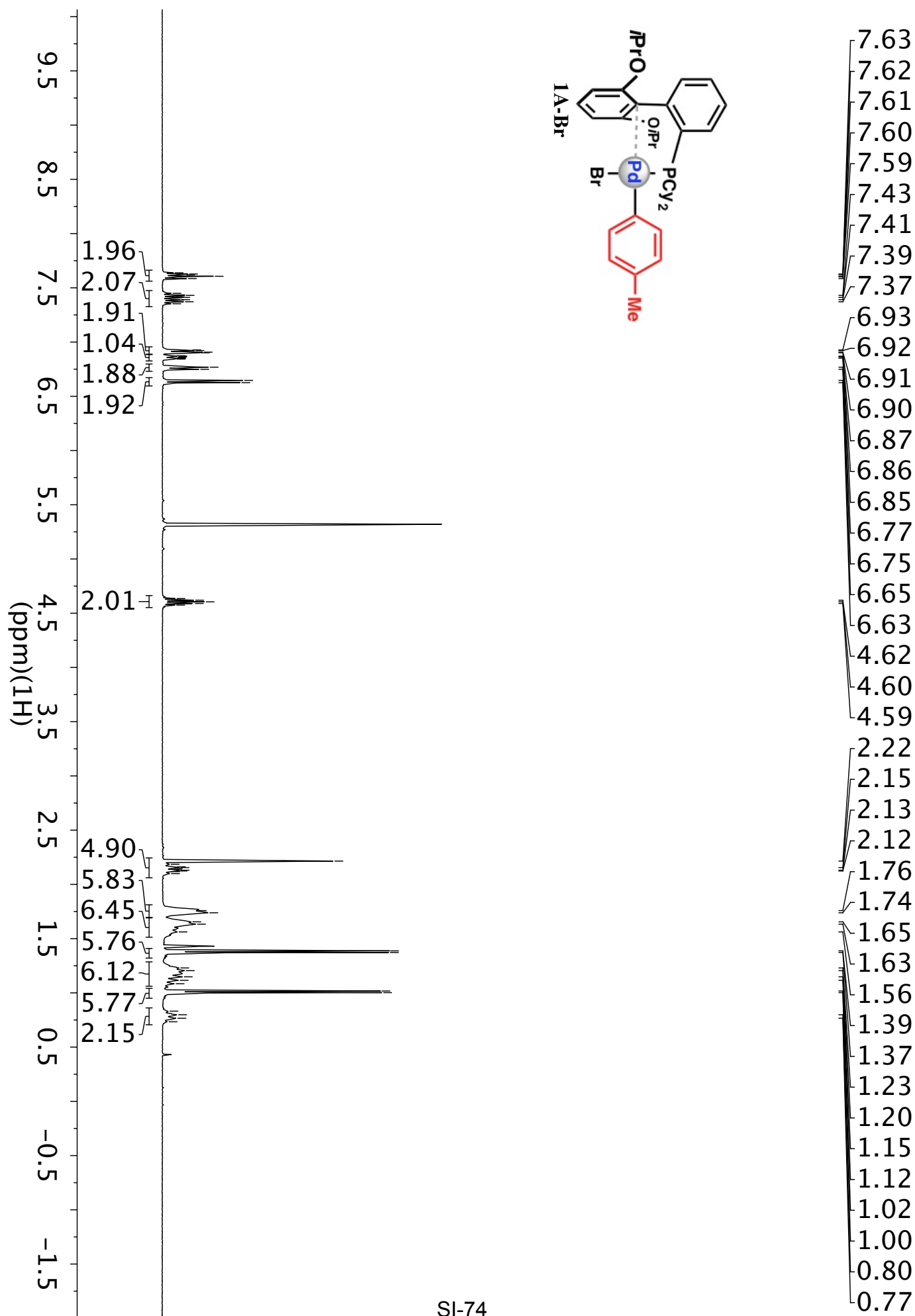


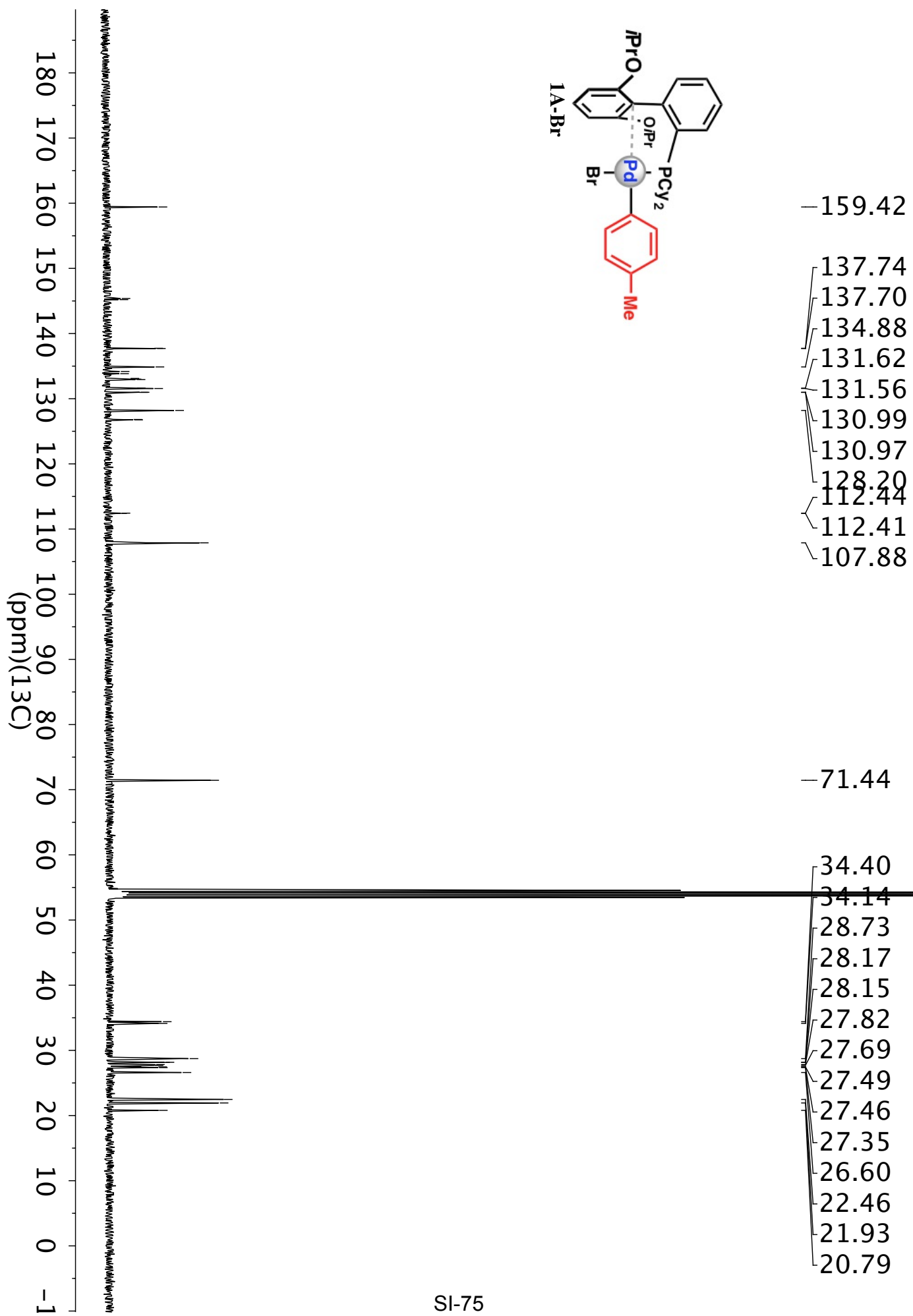




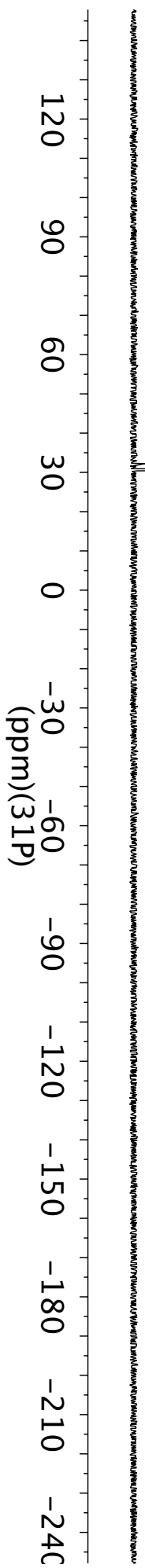
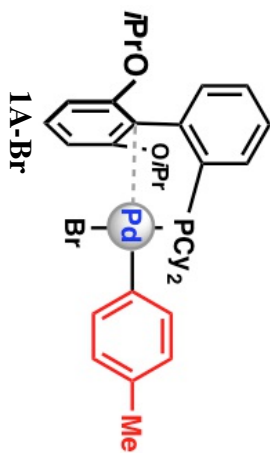
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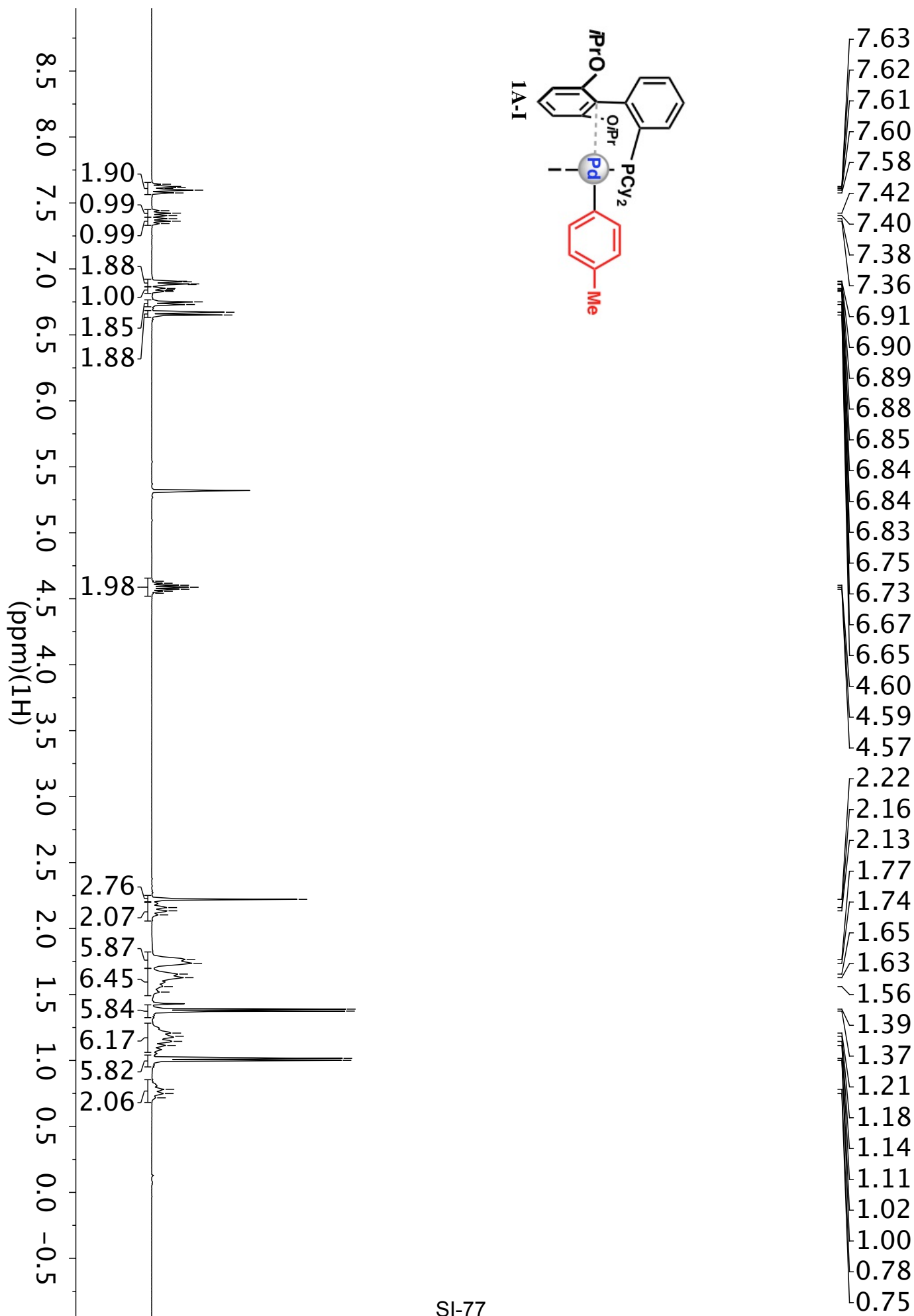






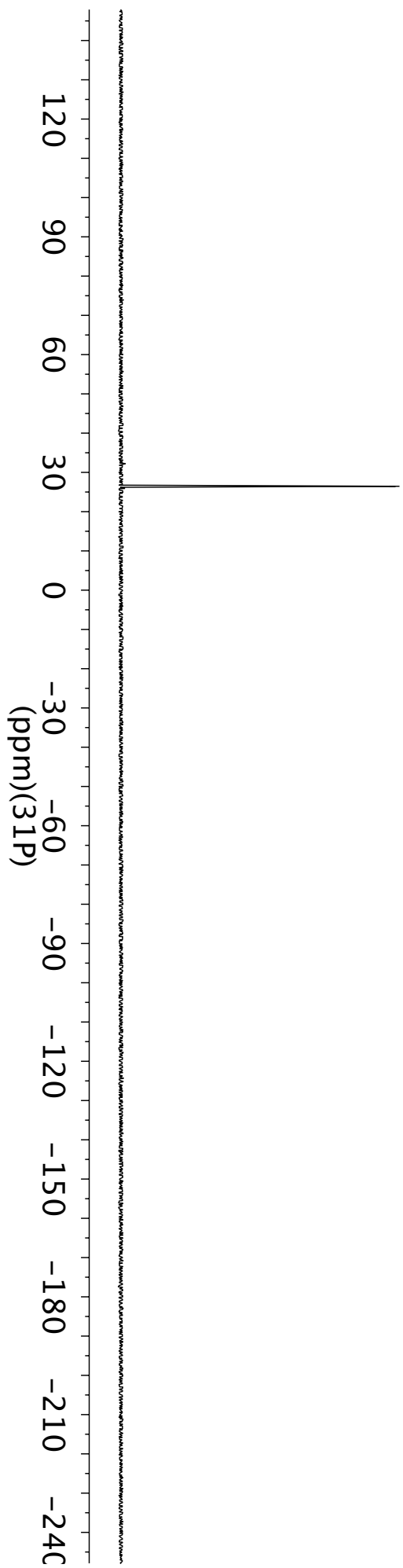
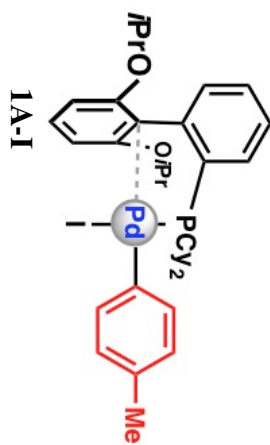
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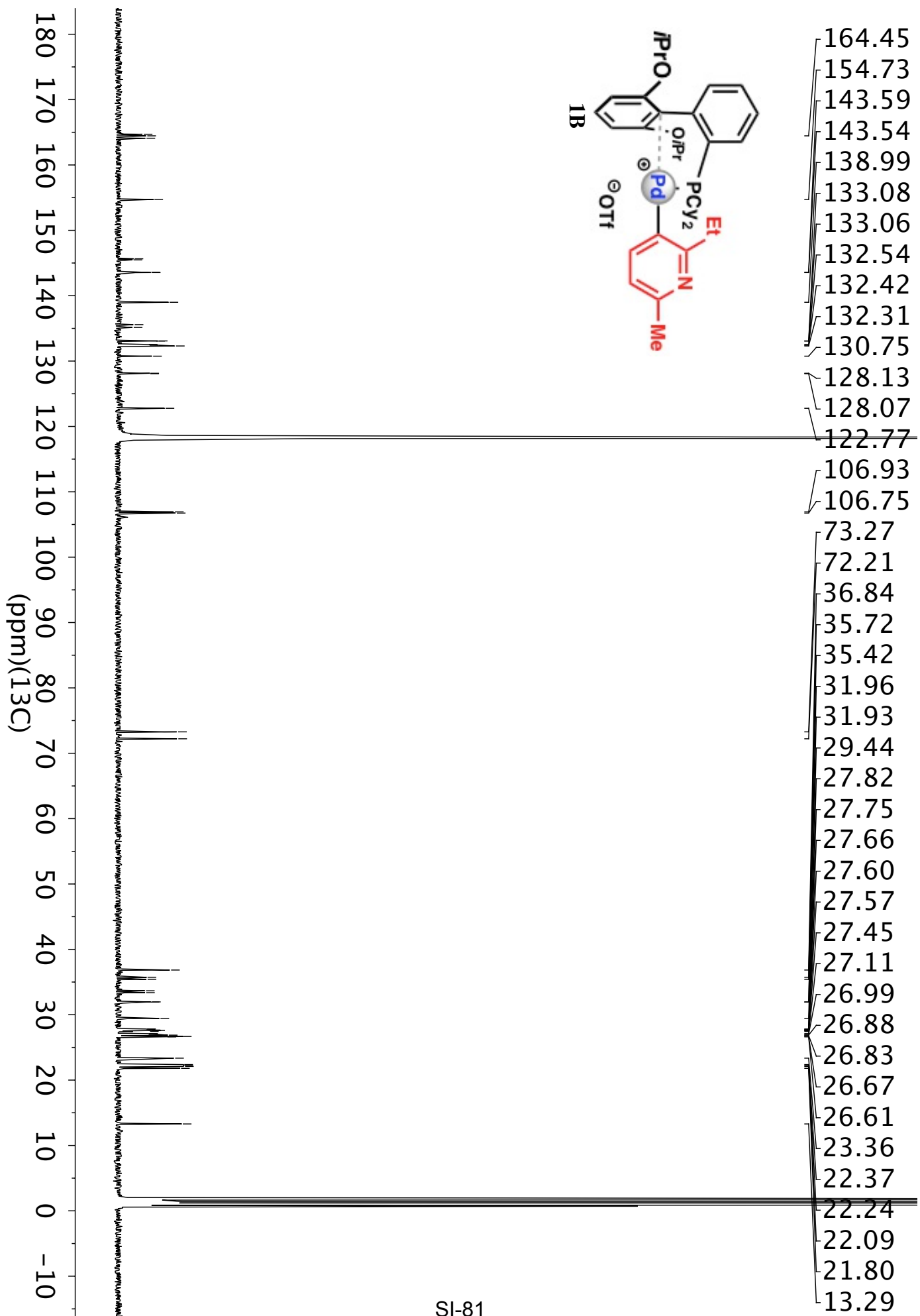
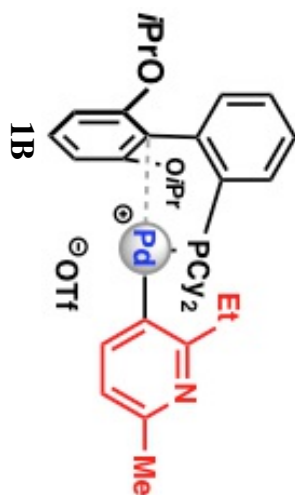


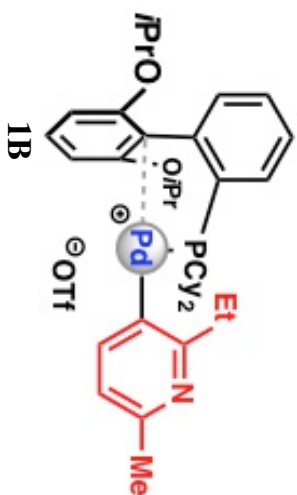


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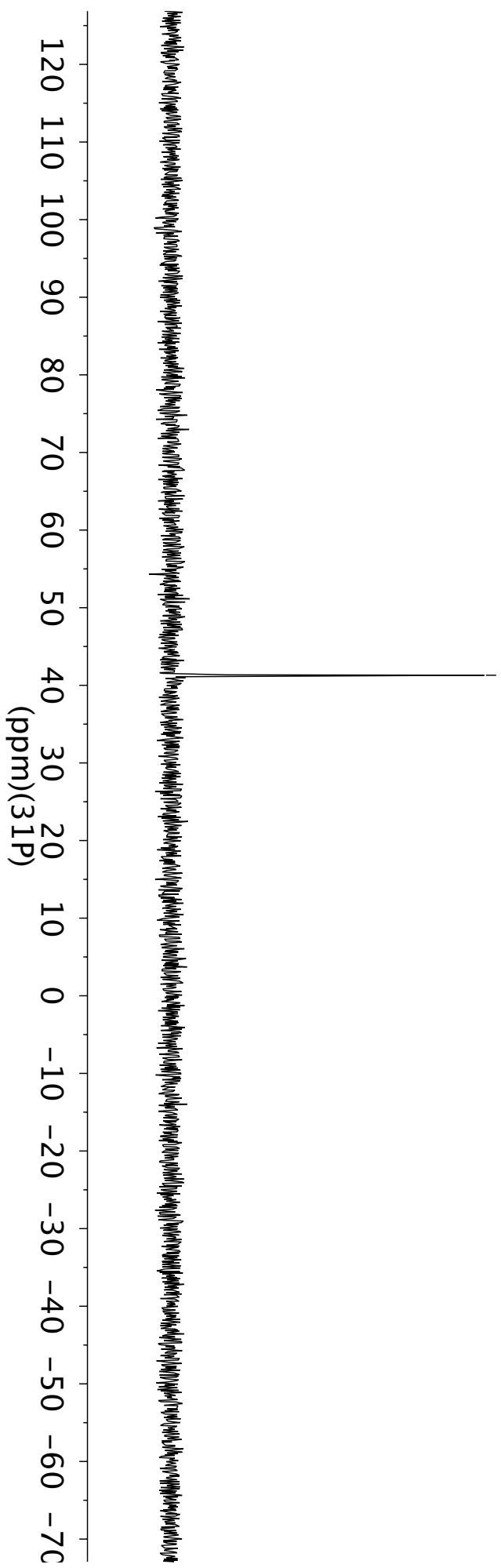




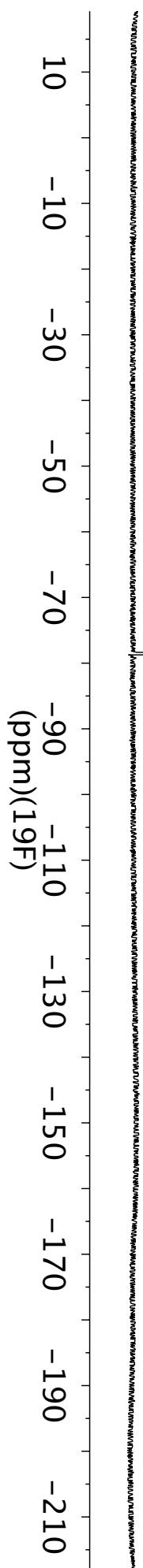
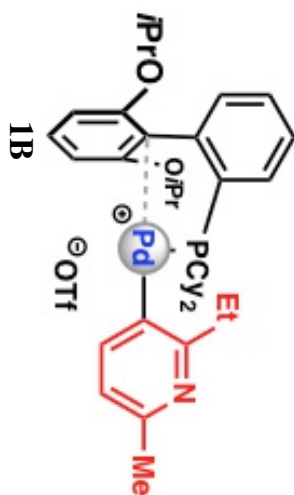


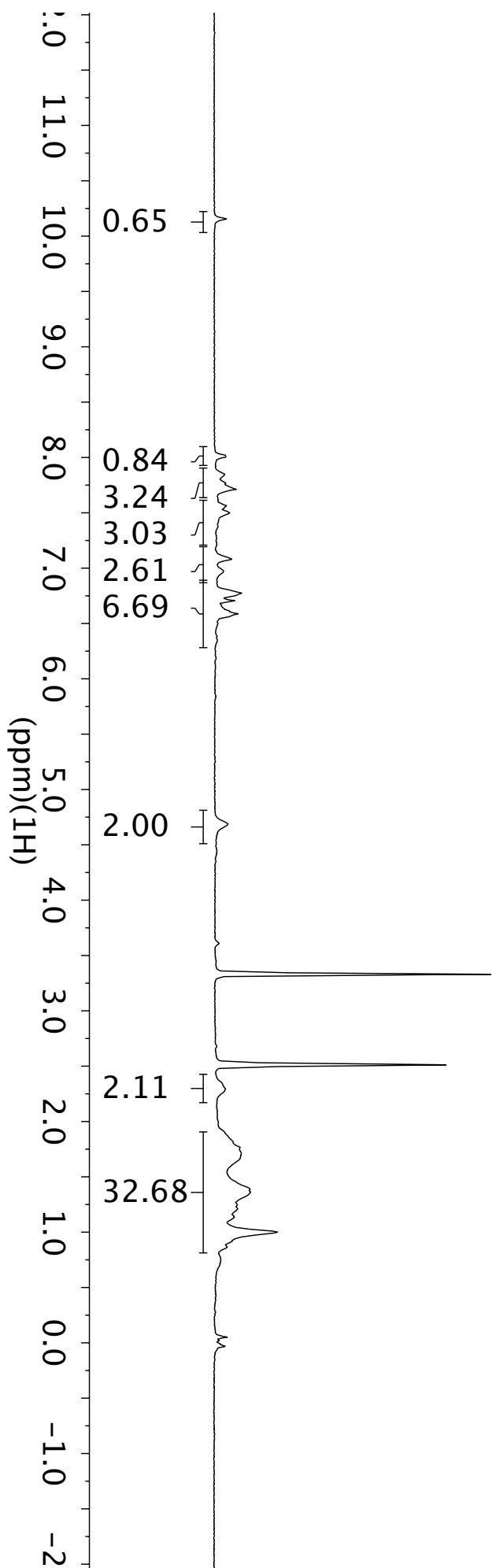
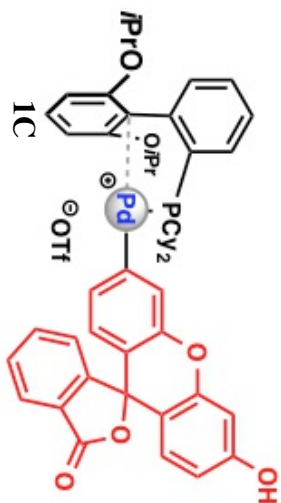


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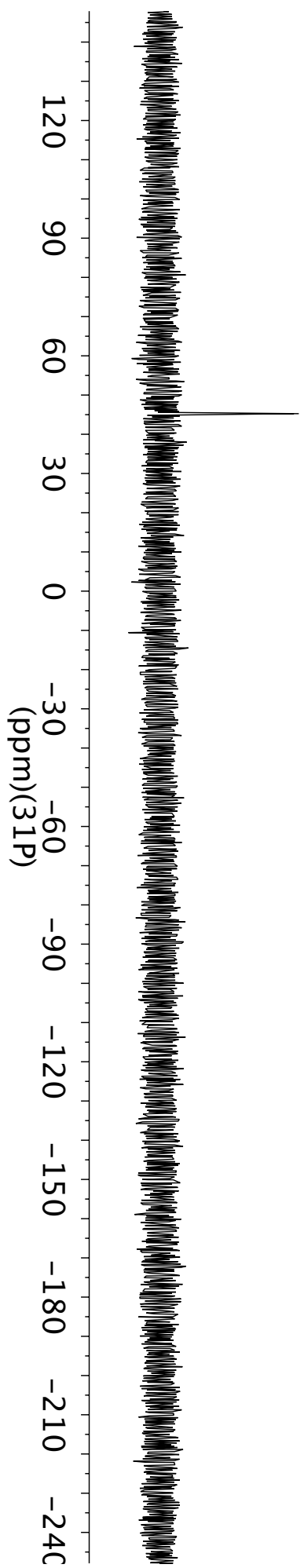
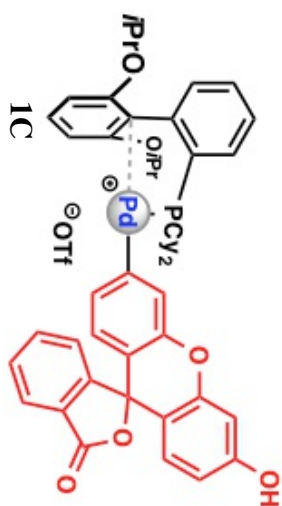


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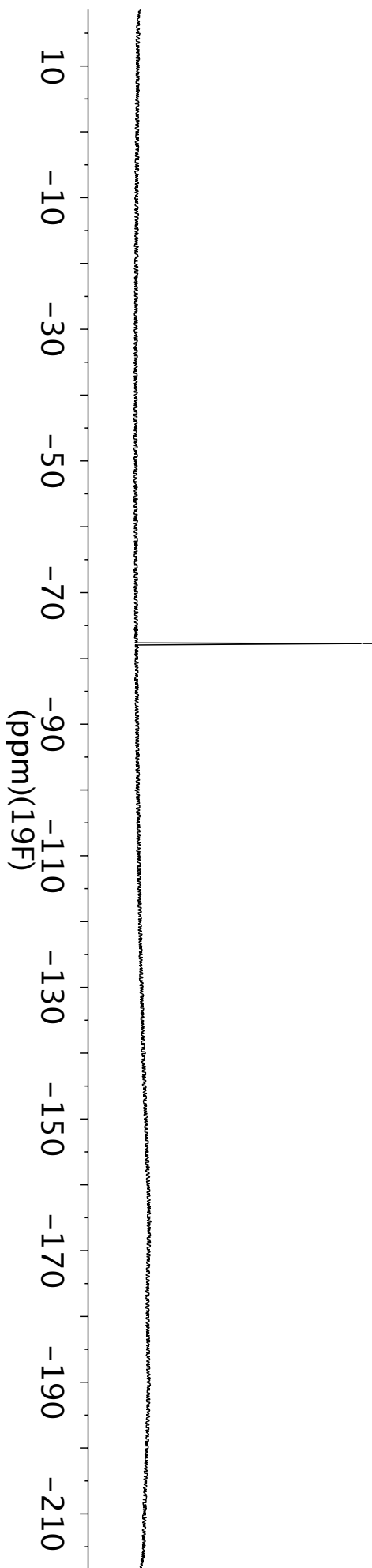
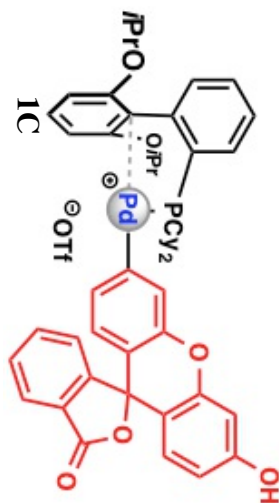




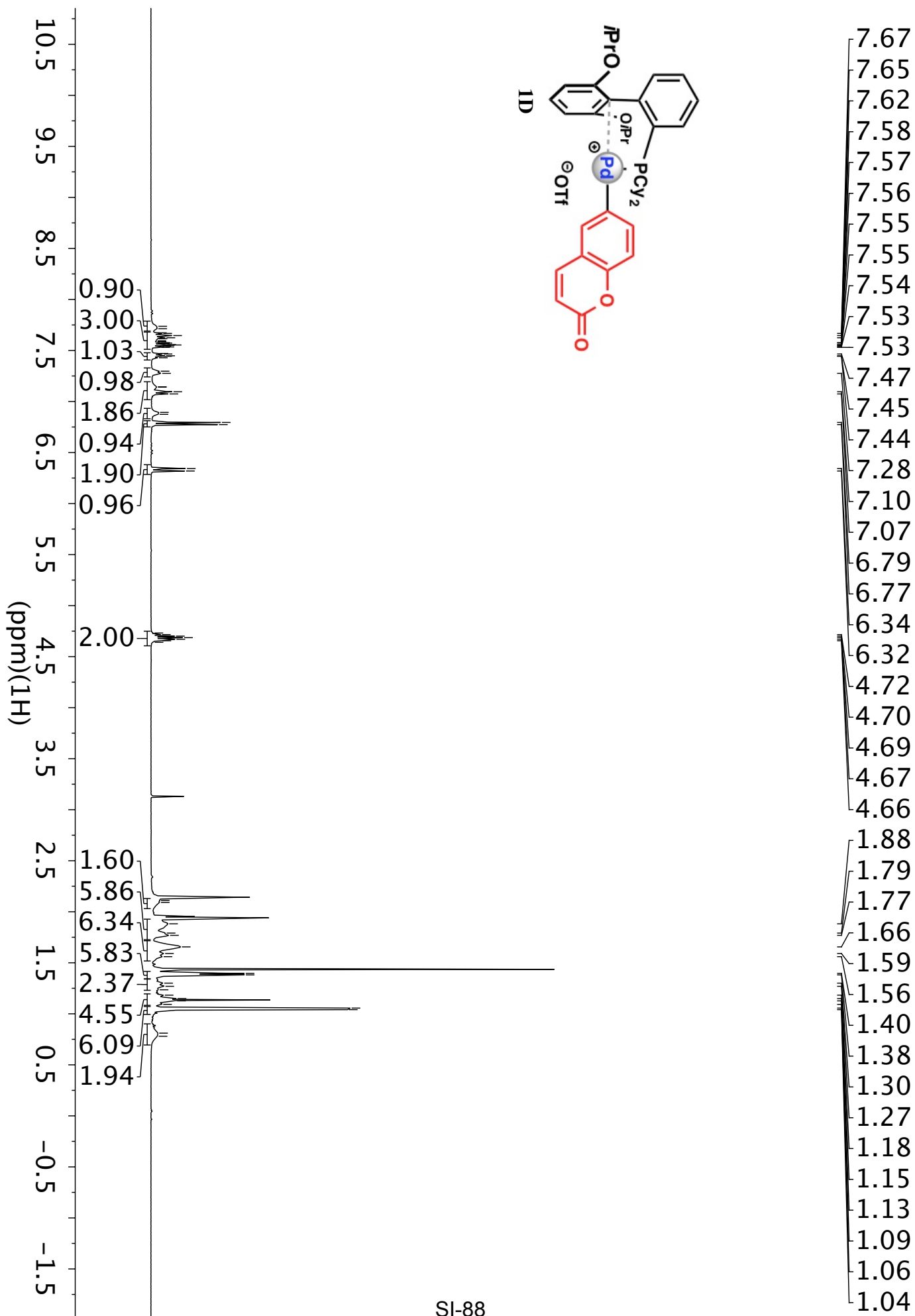
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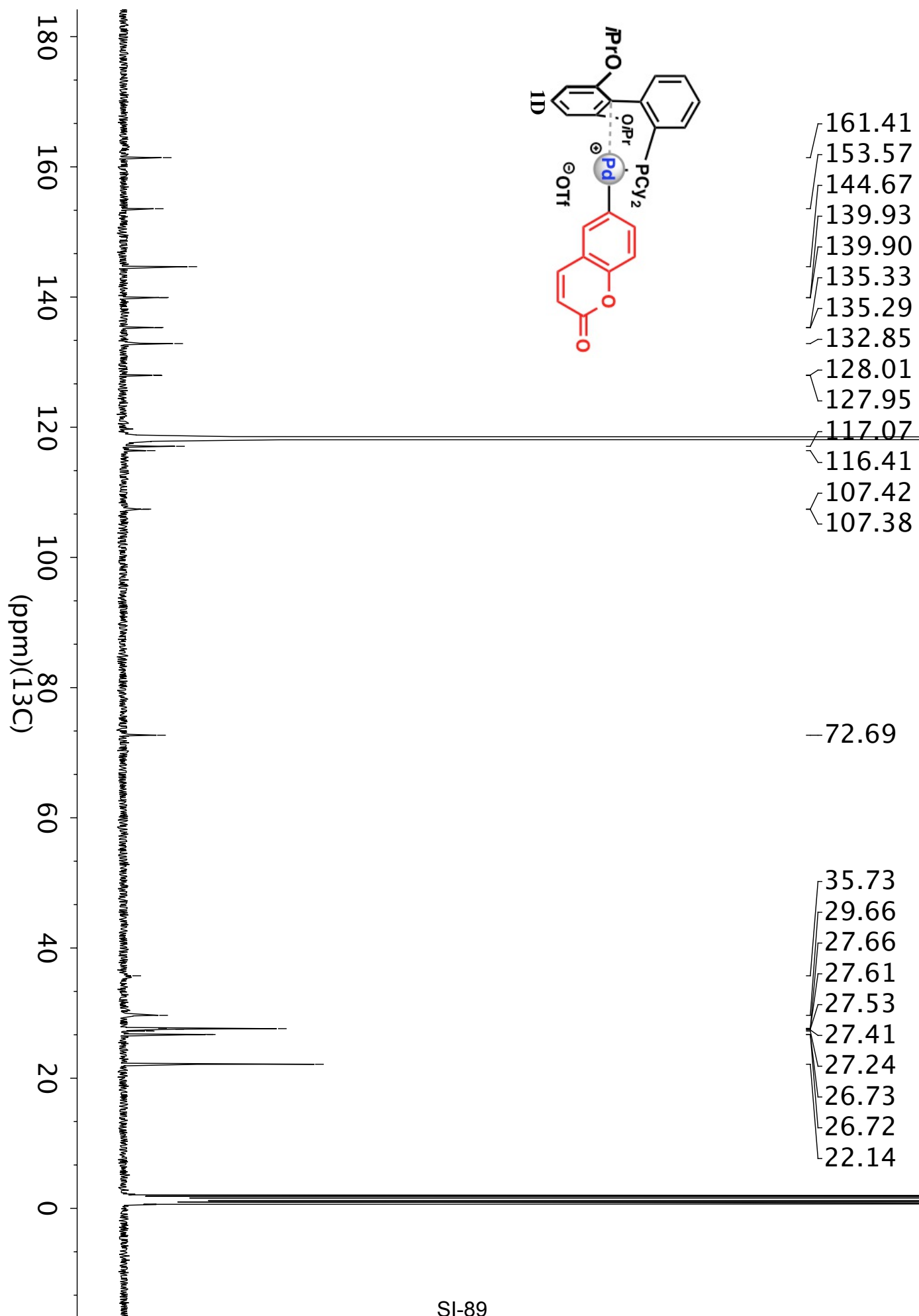


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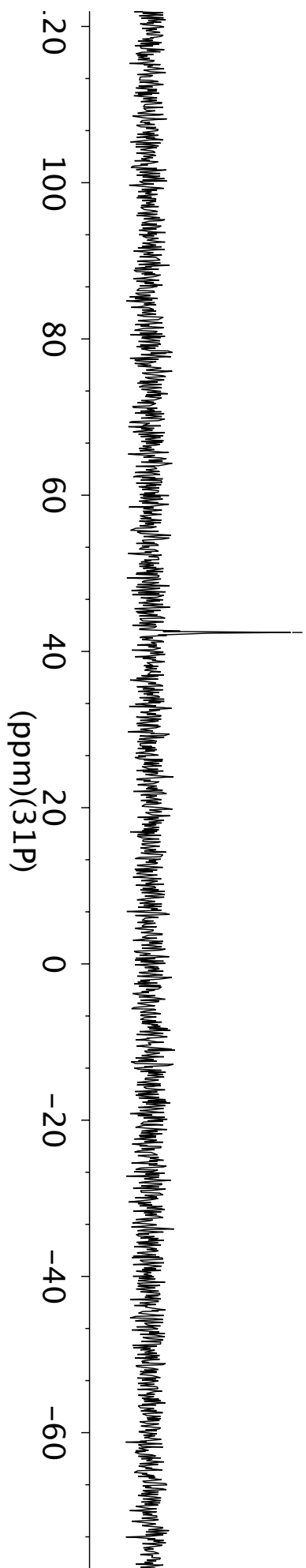
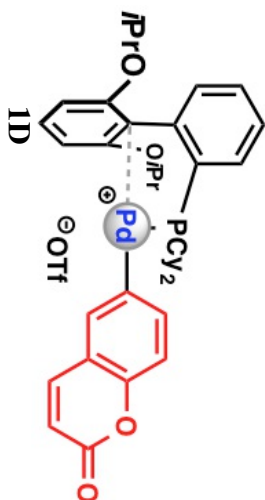




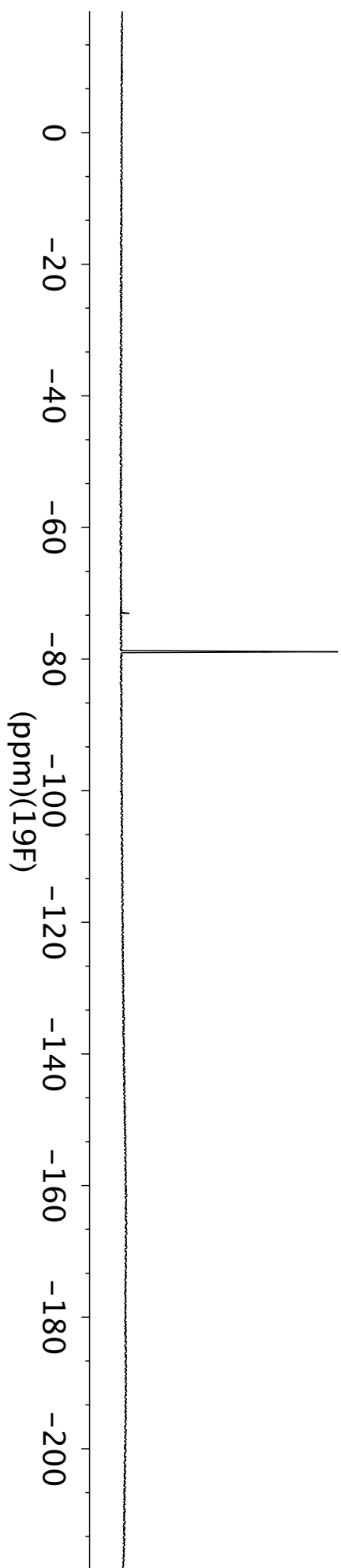
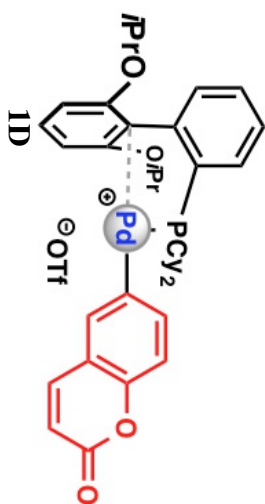


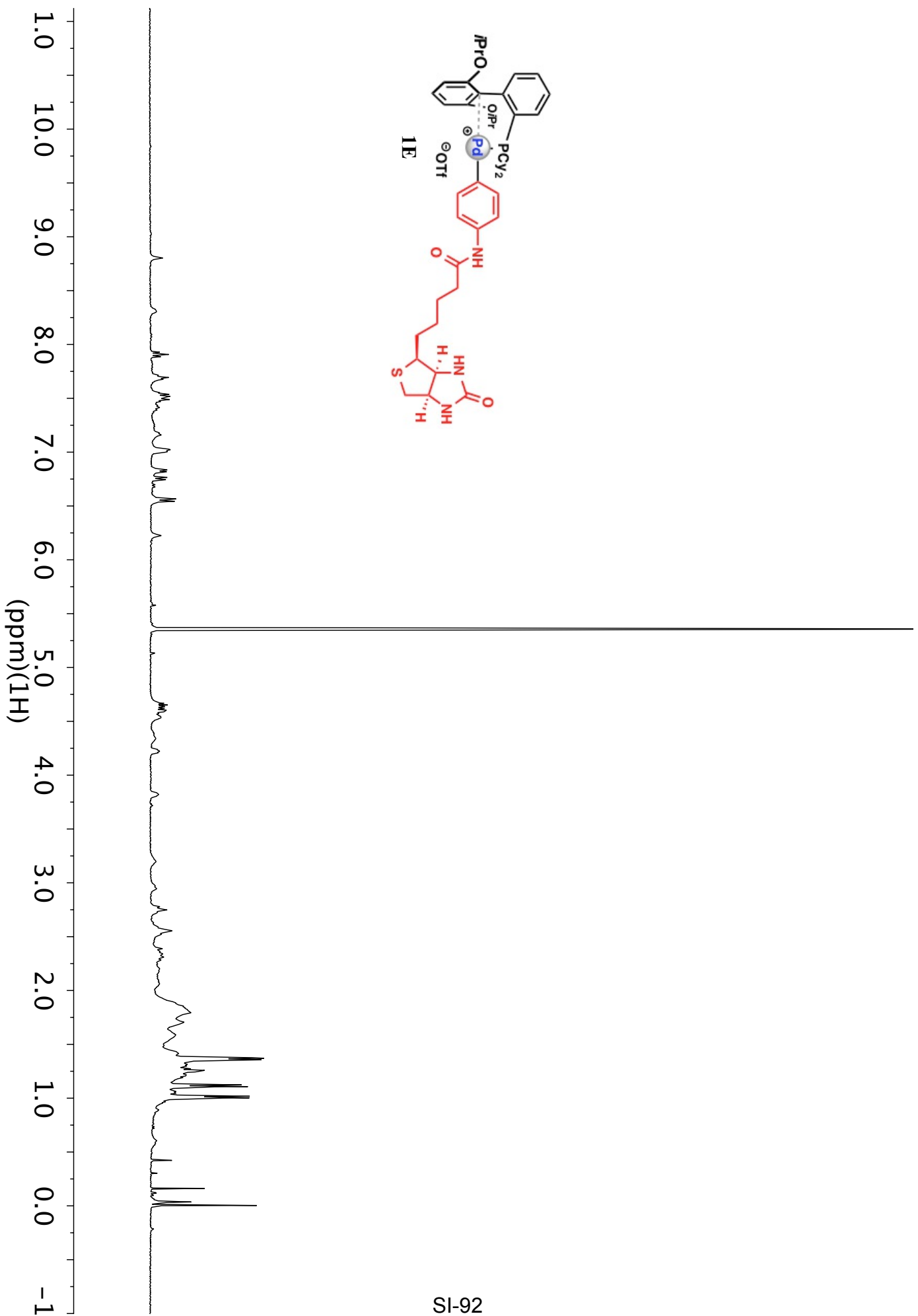
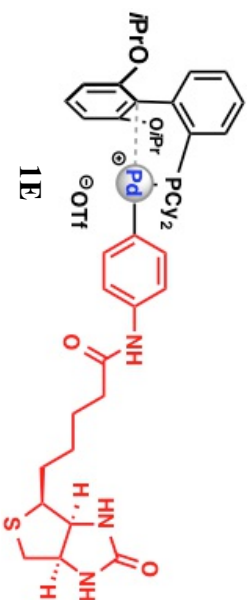


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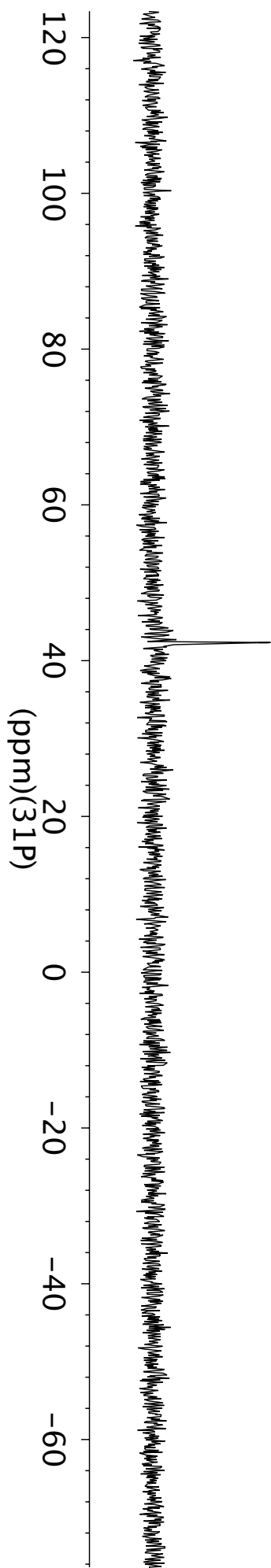
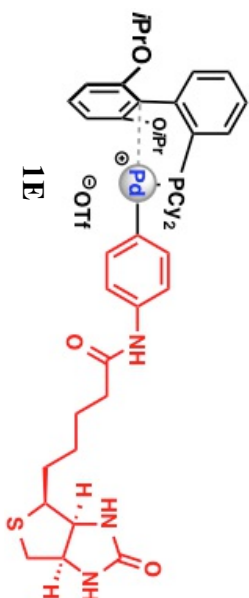


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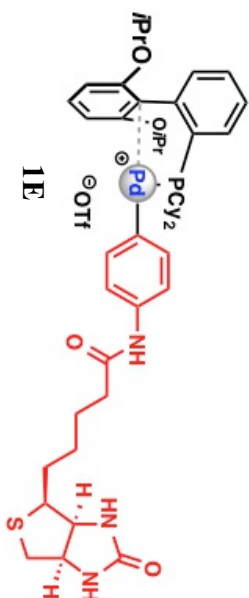




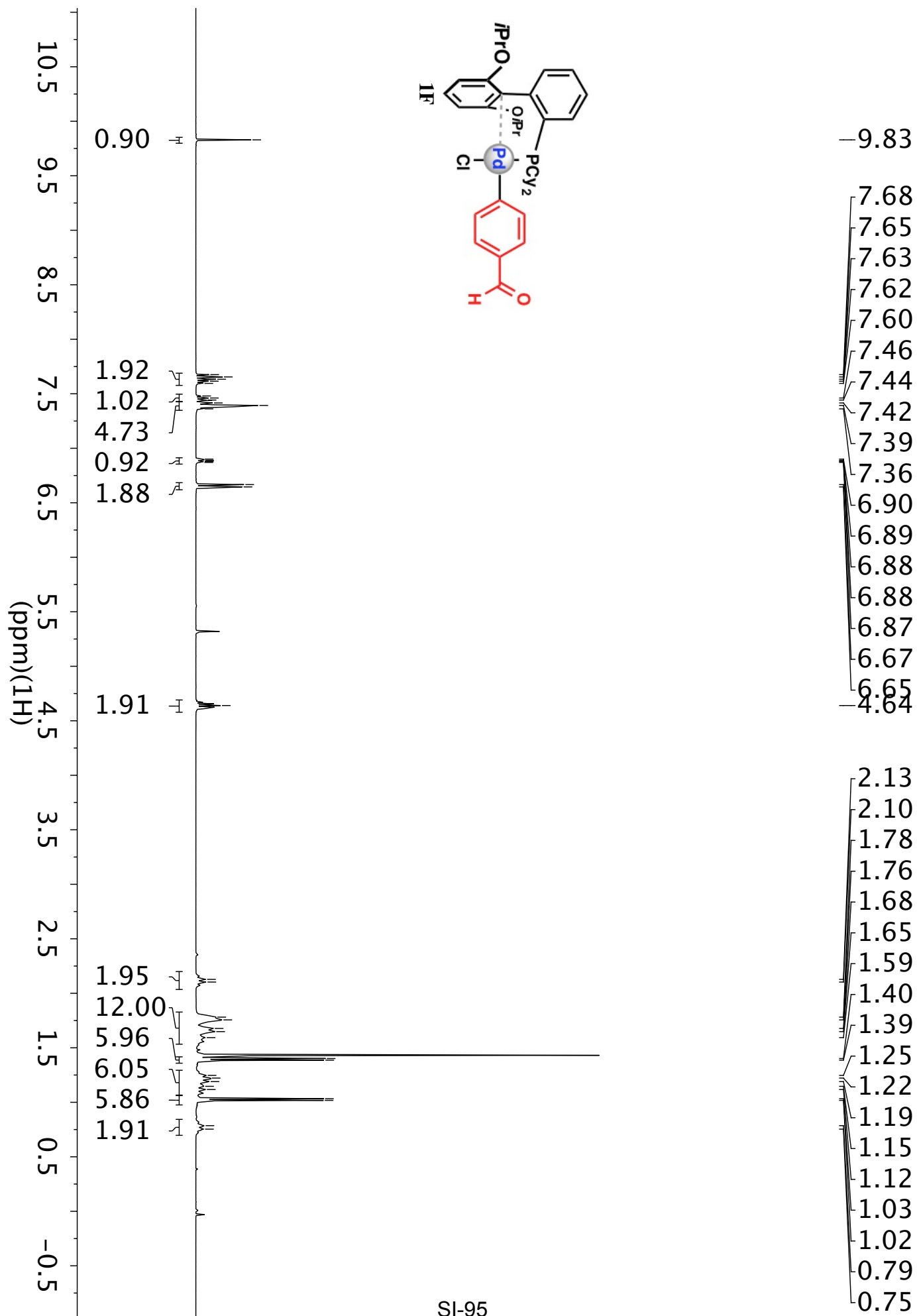
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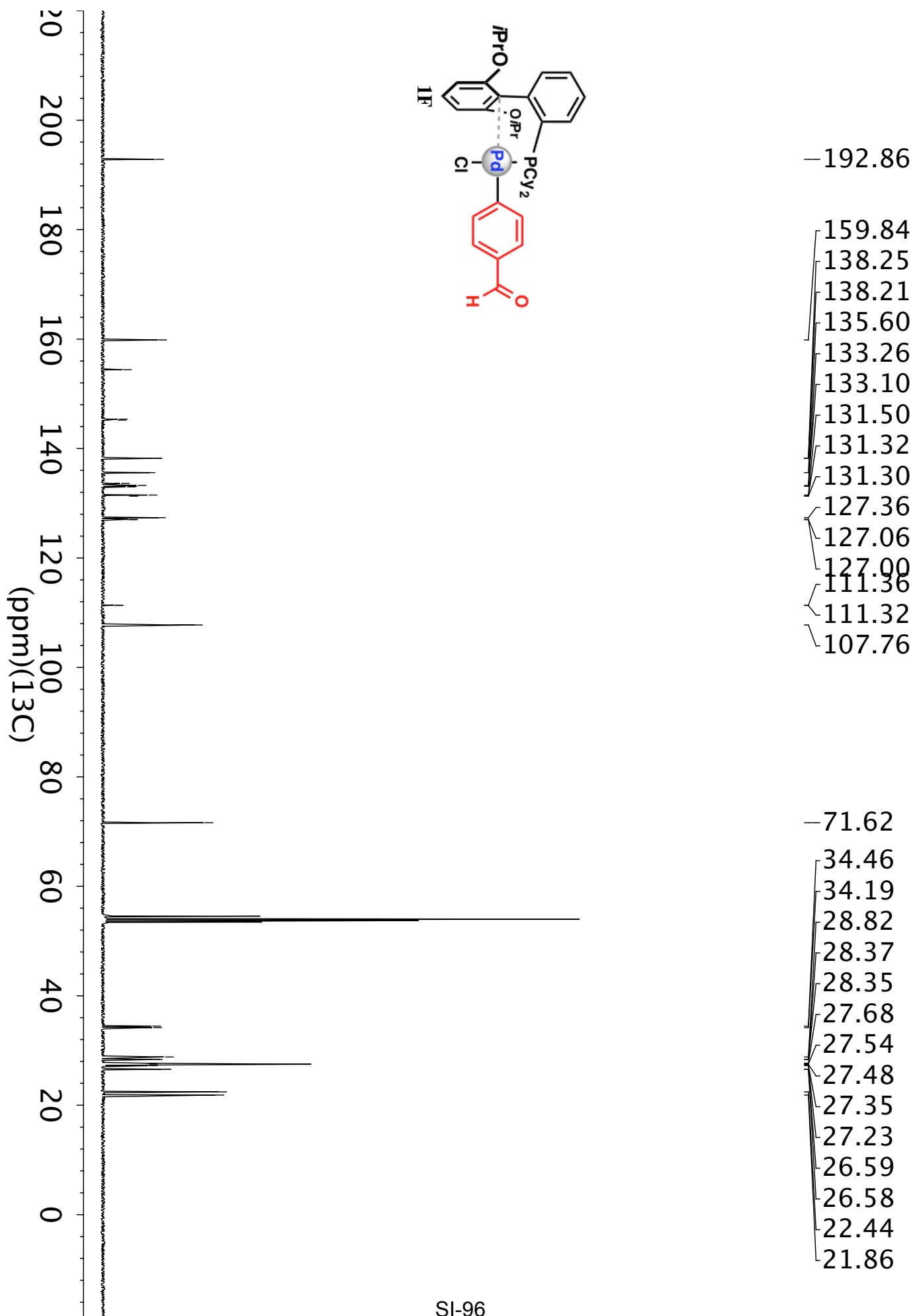


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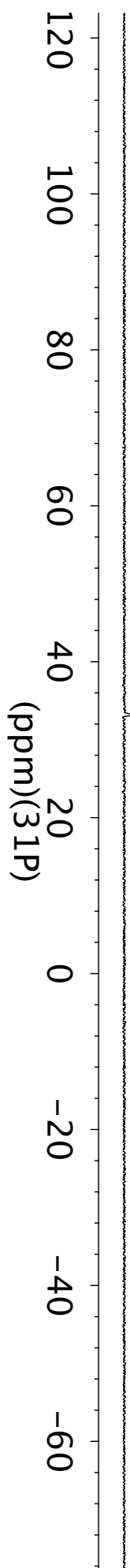
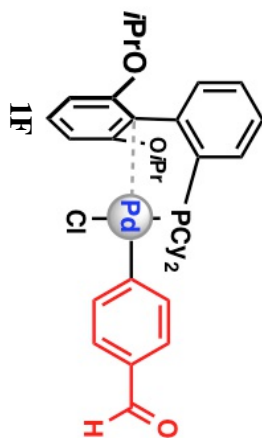


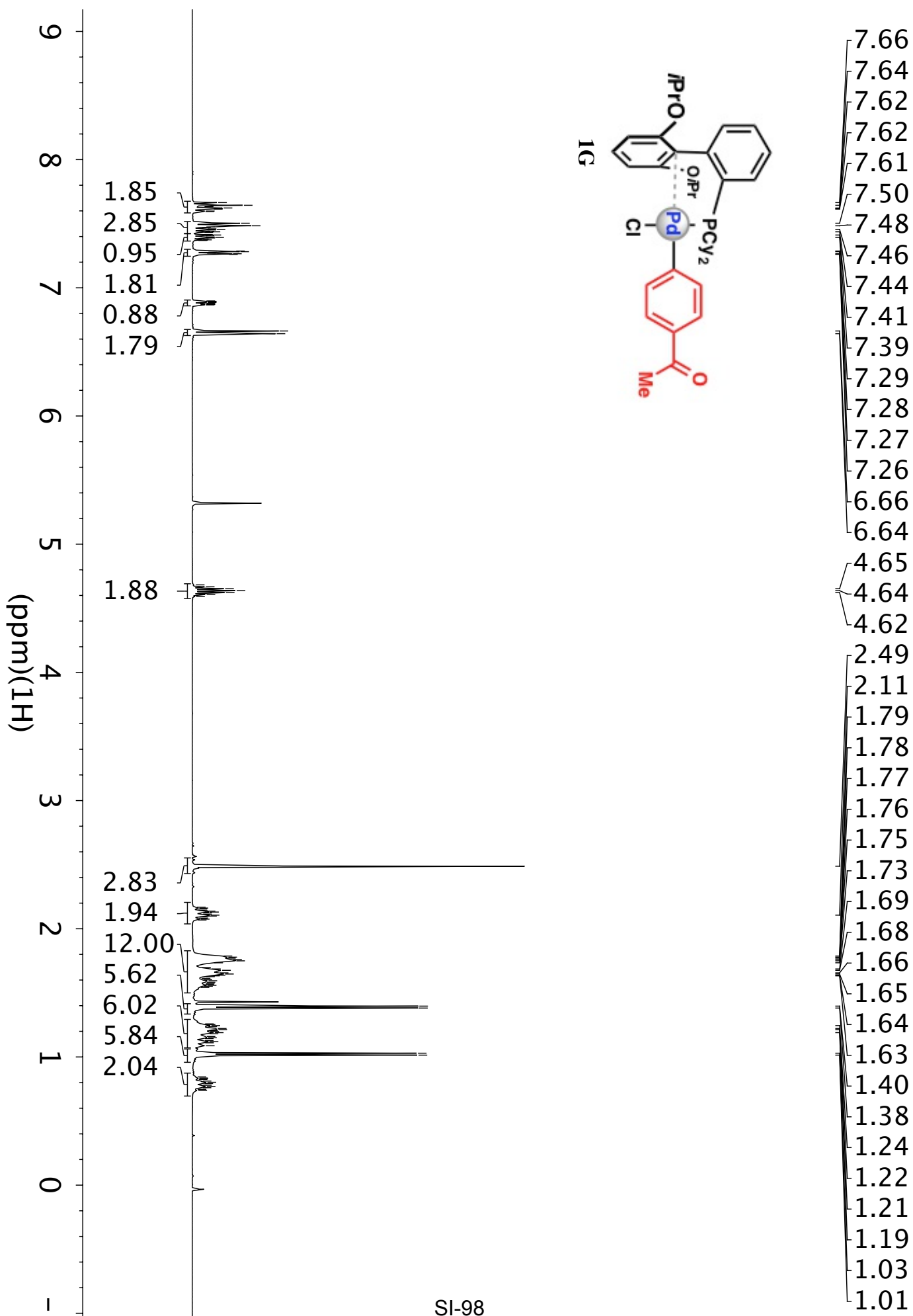
150 100 50 0 -50 -100 -150  
(ppm)(<sup>19</sup>F)

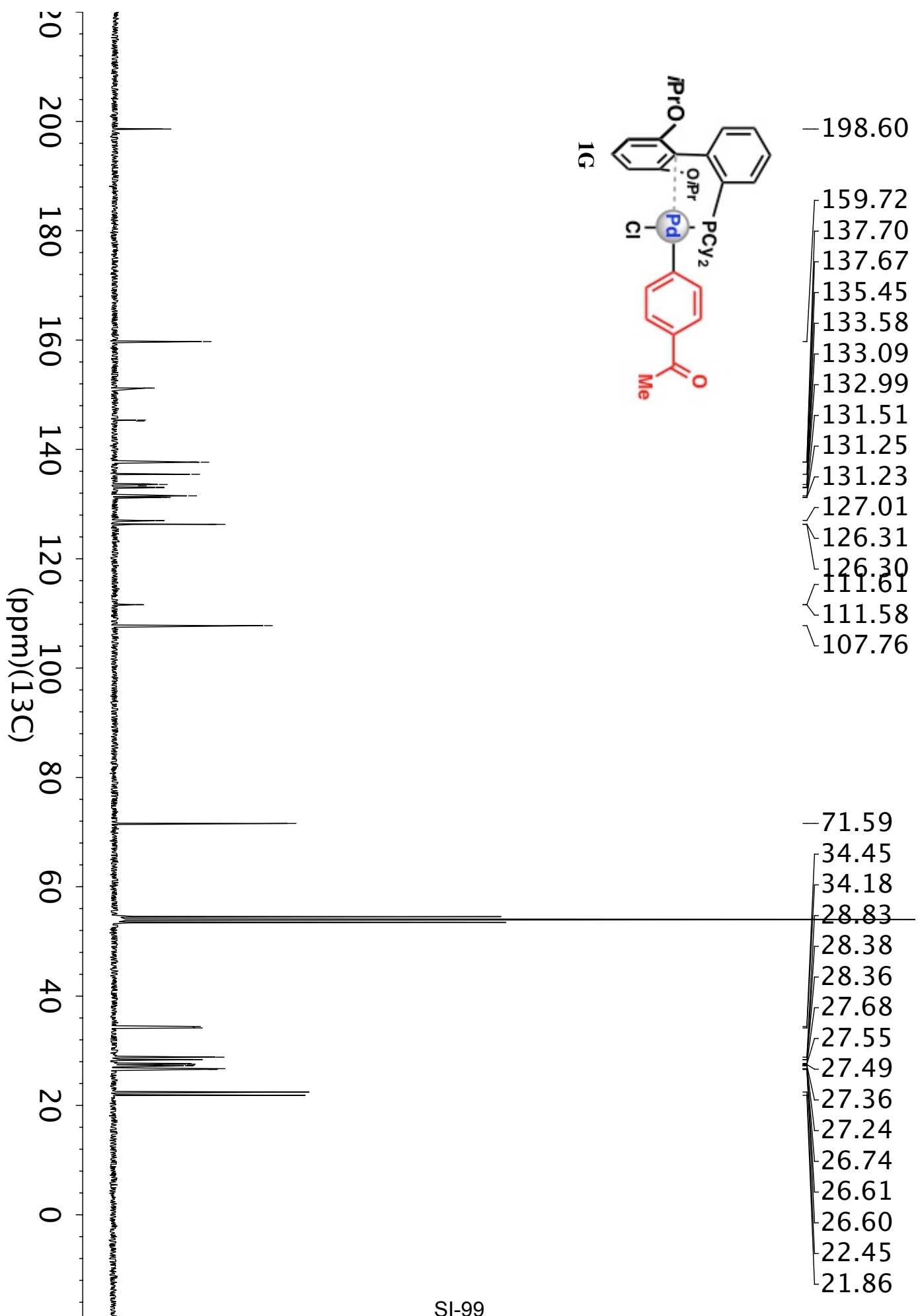




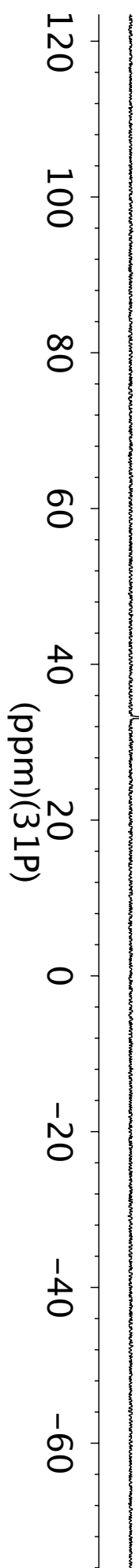
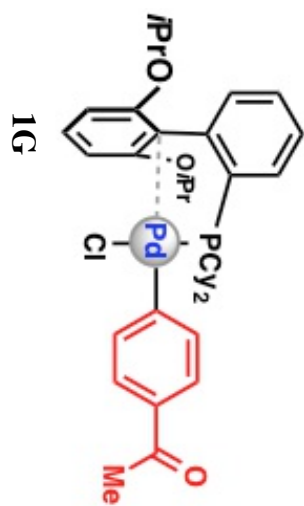
-33.19

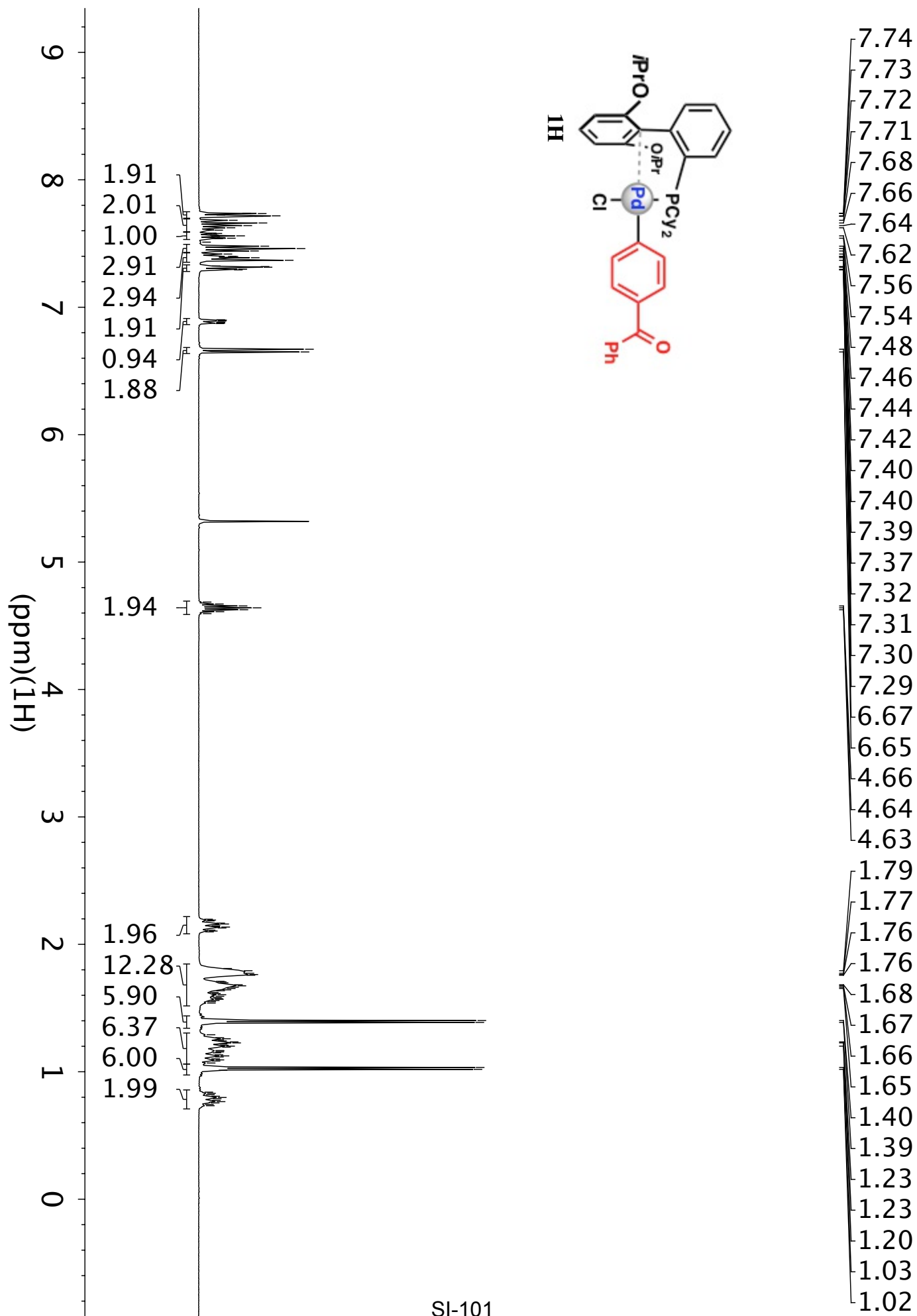






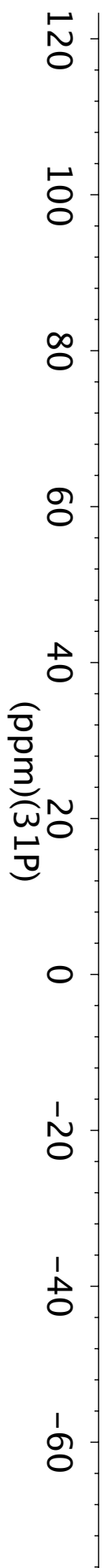
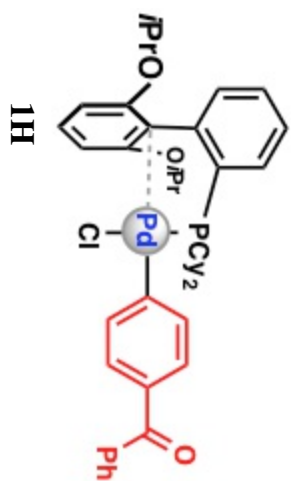
-33.19

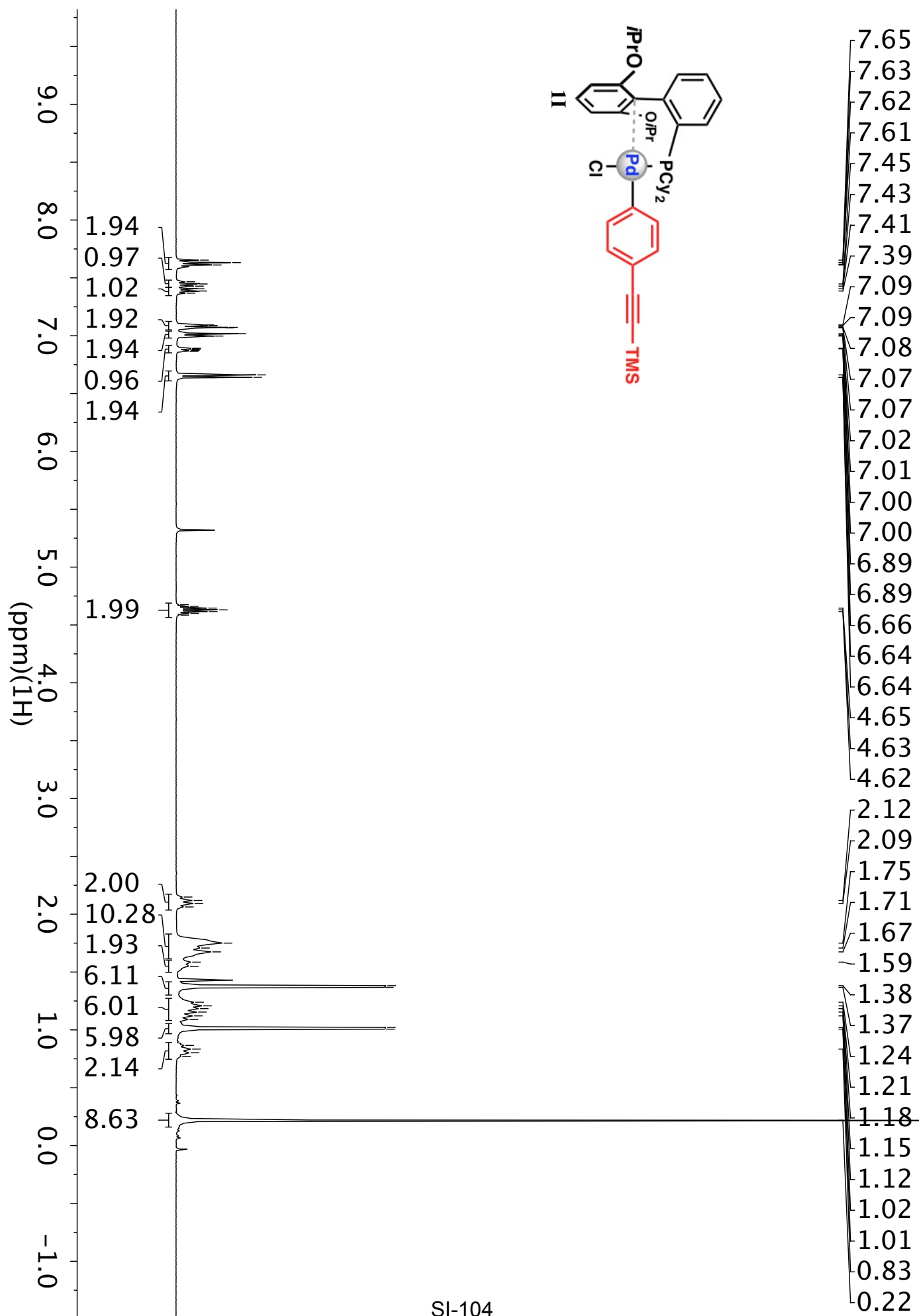






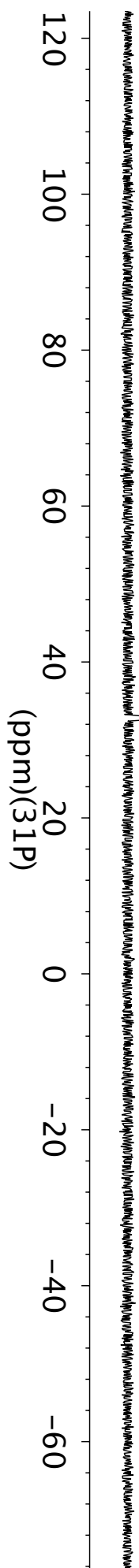
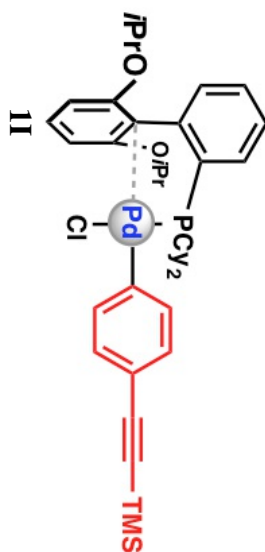
-33.29

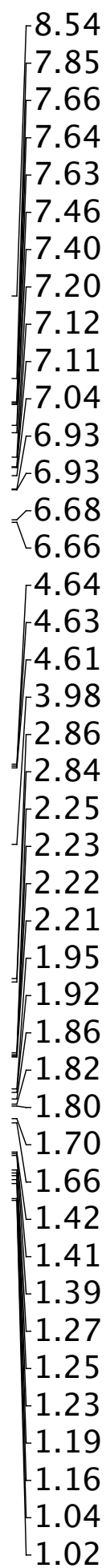
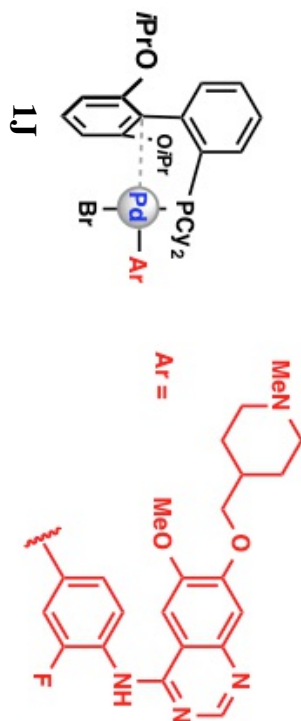
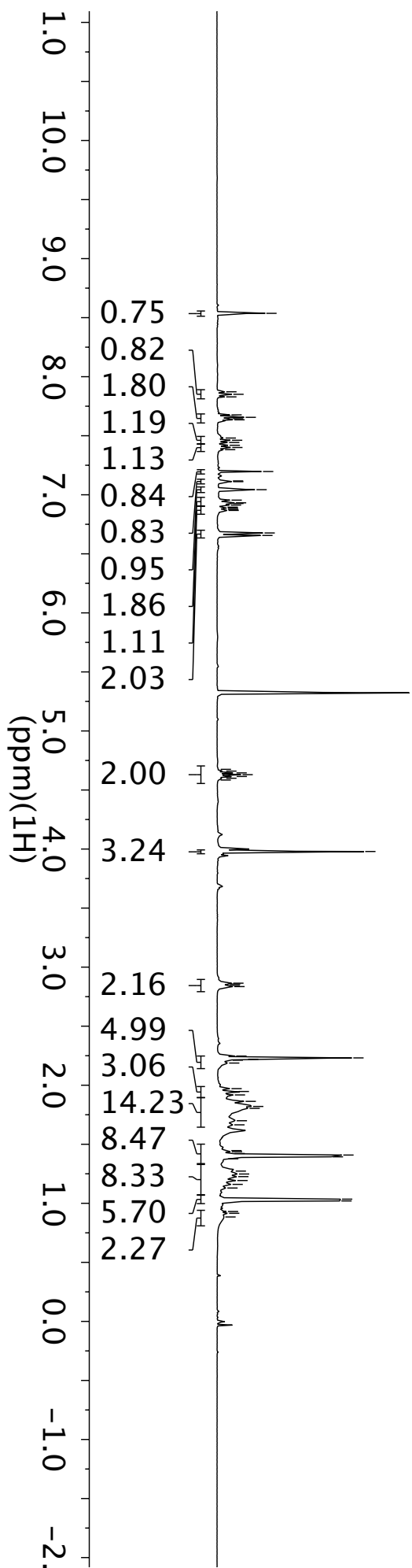


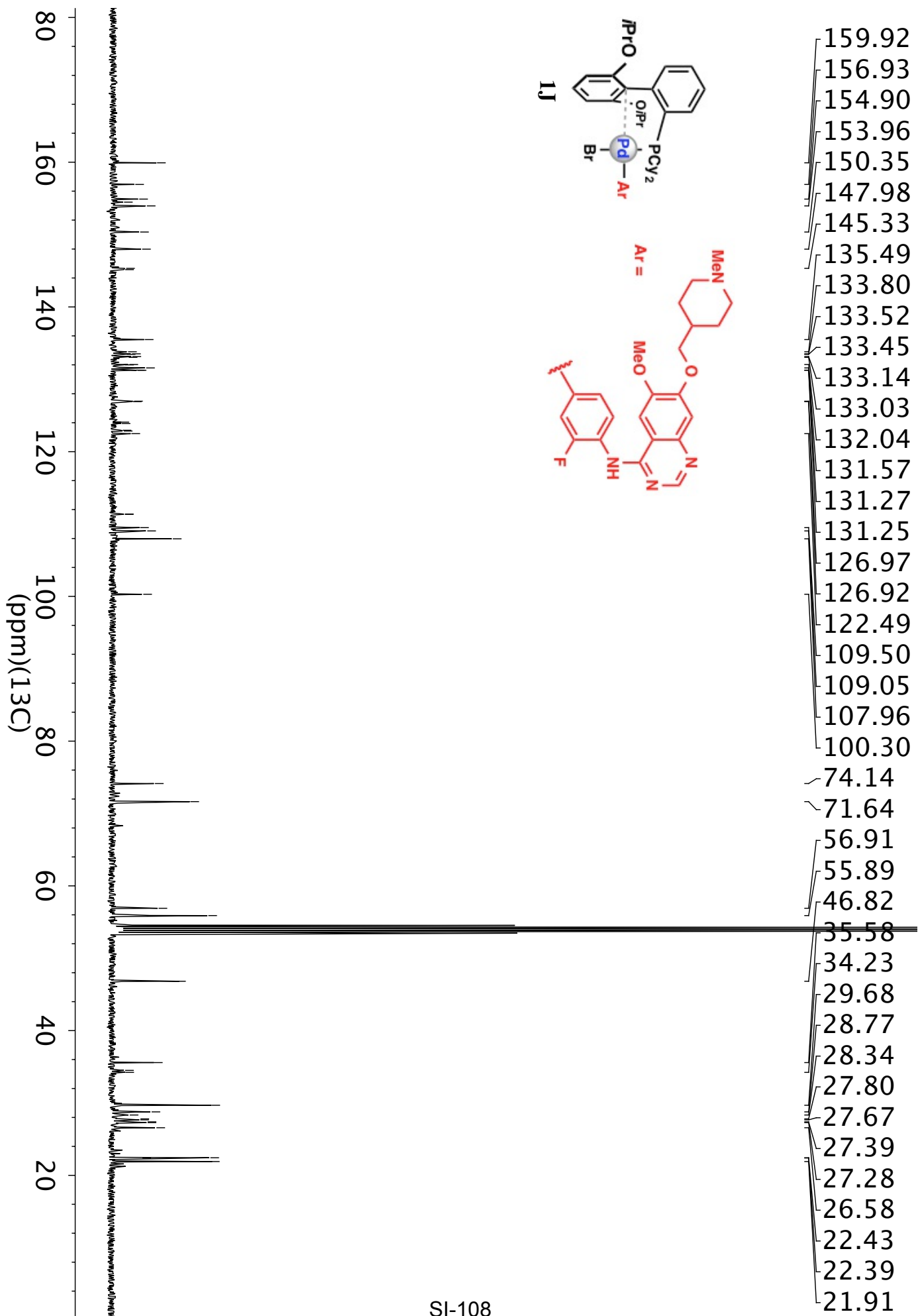




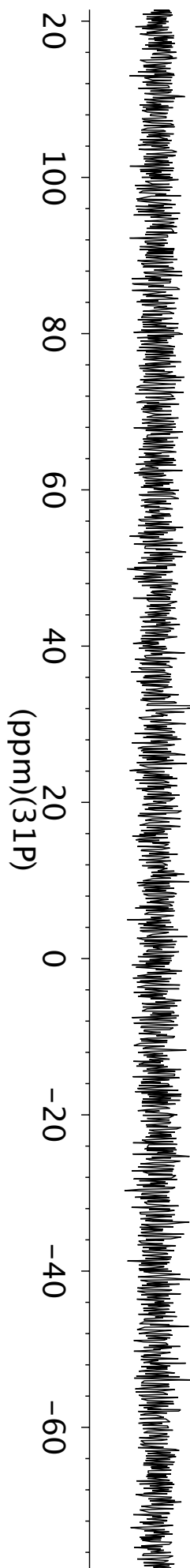
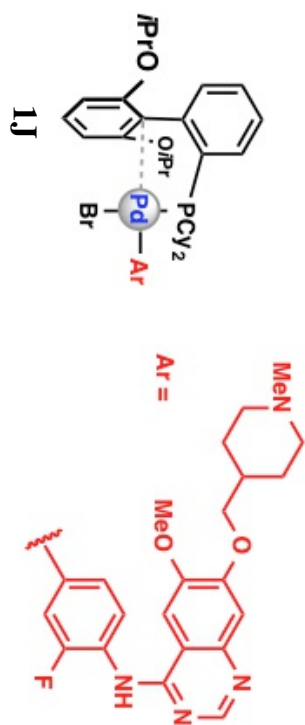
-32.69

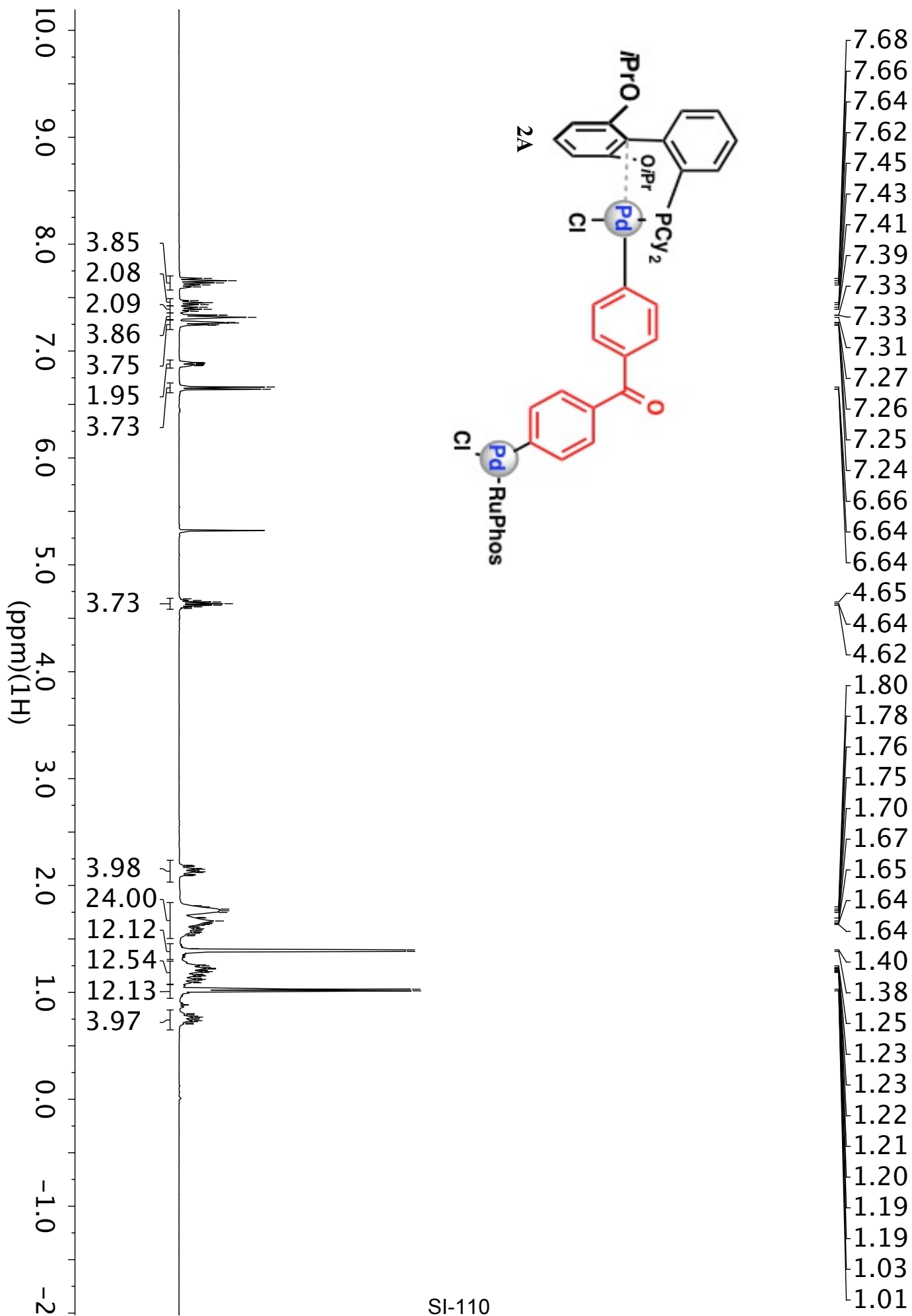


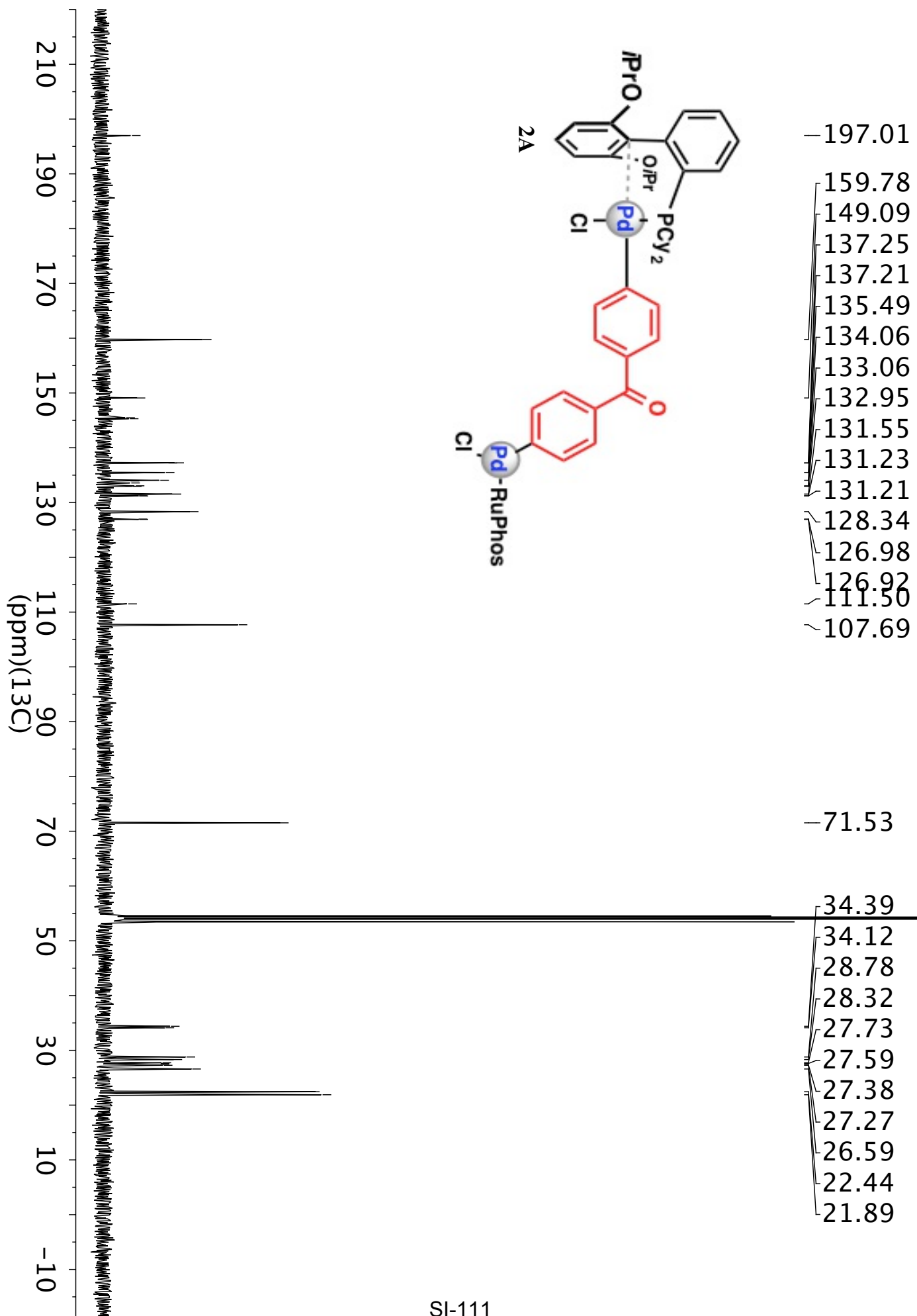




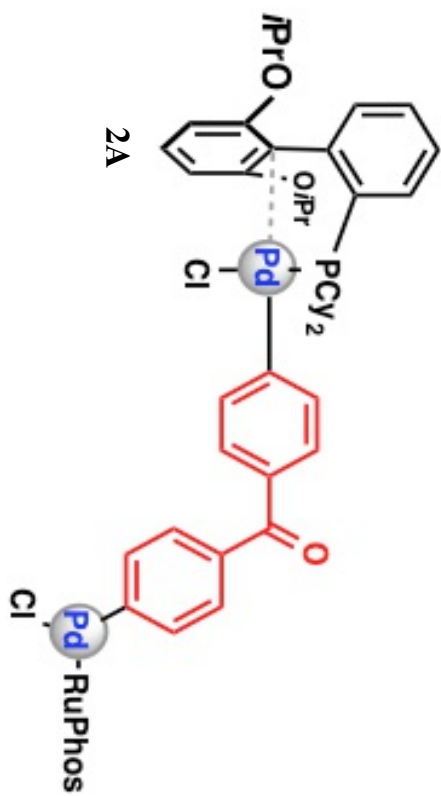
-32.24





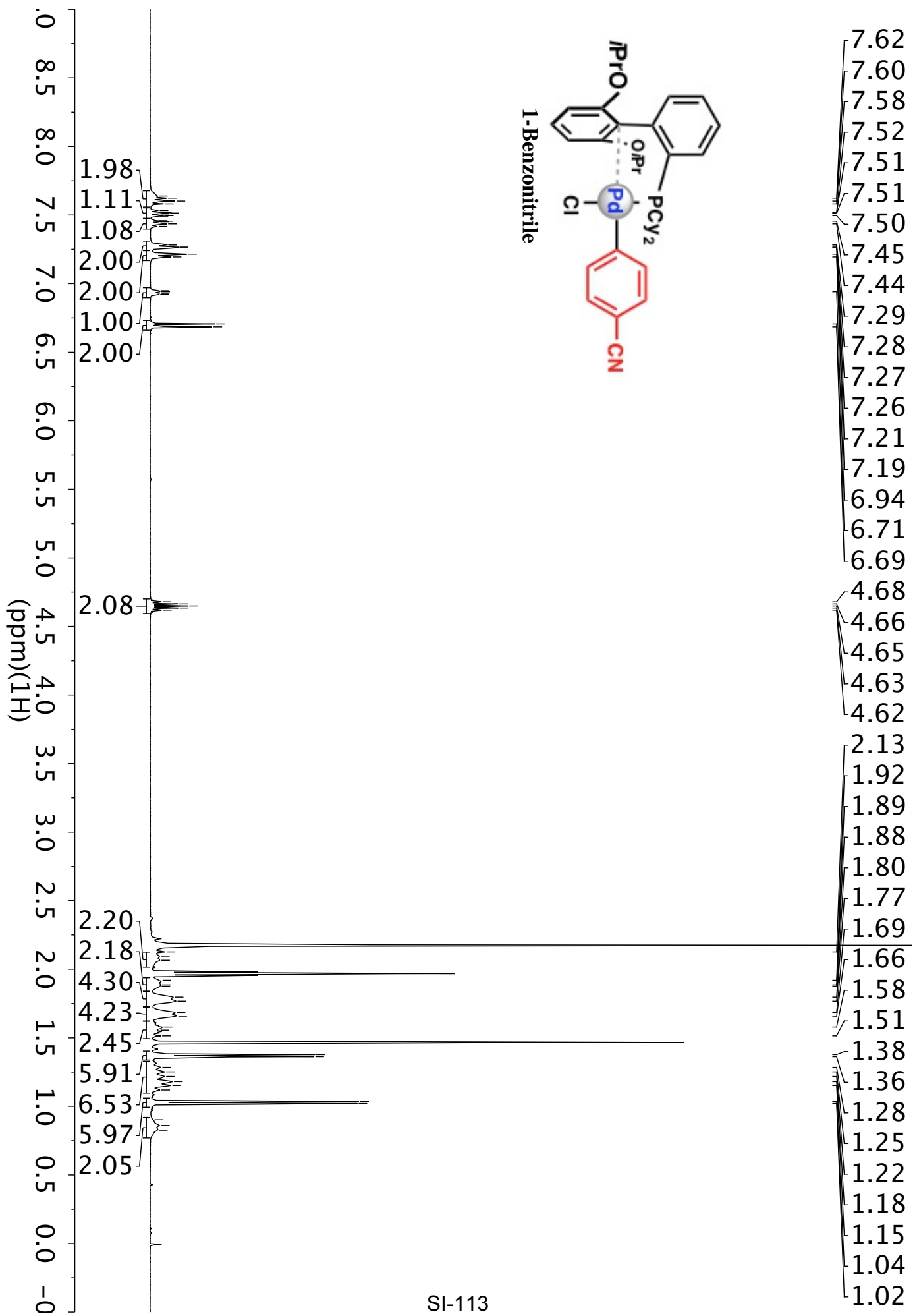
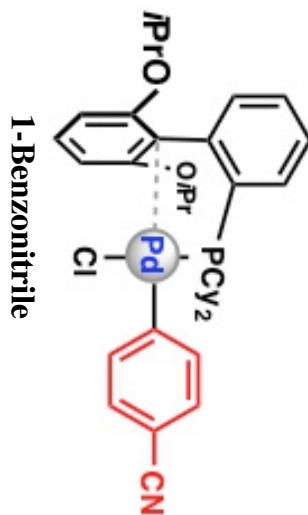


-33.27



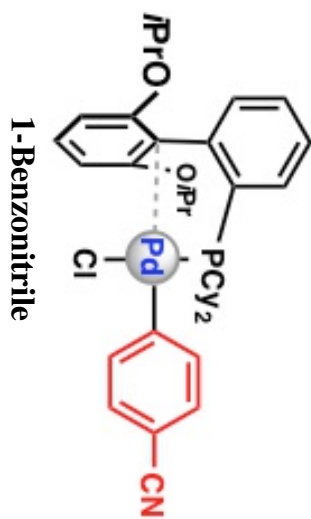
2A







-33.67





29.18  
28.93

29.17  
28.88

